```
=> d his ful

     FILE 'WPIX' ENTERED AT 12:32:50 ON 03 JUL 2006
L1          141 SEA ABB=ON  PLU=ON  MD2 OR MD 2
L2         3272 SEA ABB=ON  PLU=ON  ENDOTOXIN# OR ENDO (2W) TOXIN#
L3            6 SEA ABB=ON  PLU=ON  L1 AND L2
                D TI 1-6
                D .WP 4
L4        10586 SEA ABB=ON  PLU=ON  TOXIN#
L5            1 SEA ABB=ON  PLU=ON  L4 AND L1
L6            6 SEA ABB=ON  PLU=ON  L5 OR L3
L7          355 SEA ABB=ON  PLU=ON  WEISS J?/AU
L8            1 SEA ABB=ON  PLU=ON  GIOANNINI T?/AU
L9            1 SEA ABB=ON  PLU=ON  TEGHANEMT A?/AU
L10         316 SEA ABB=ON  PLU=ON  SUBRAMANIAN R?/AU
L11         670 SEA ABB=ON  PLU=ON  (L7 OR L8 OR L9 OR L10)
L12           1 SEA ABB=ON  PLU=ON  L11 AND L1
L13           2 SEA ABB=ON  PLU=ON  L11 AND L2
L14           2 SEA ABB=ON  PLU=ON  L12 OR L13
L15           1 SEA ABB=ON  PLU=ON  L14 NOT L3
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=> fil wpix
FILE 'WPIX' ENTERED AT 12:35:47 ON 03 JUL 2006
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FILE LAST UPDATED:          29 JUN 2006      <20060629/UP>
MOST RECENT DERWENT UPDATE:     200641        <200641/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

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=> d que l6
L1          141 SEA FILE=WPIX ABB=ON  PLU=ON  MD2 OR MD 2
L2         3272 SEA FILE=WPIX ABB=ON  PLU=ON  ENDOTOXIN# OR ENDO (2W) TOXIN#
L3            6 SEA FILE=WPIX ABB=ON  PLU=ON  L1 AND L2
L4        10586 SEA FILE=WPIX ABB=ON  PLU=ON  TOXIN#
L5            1 SEA FILE=WPIX ABB=ON  PLU=ON  L4 AND L1
L6            6 SEA FILE=WPIX ABB=ON  PLU=ON  L5 OR L3


=> d que l15
L1          141 SEA FILE=WPIX ABB=ON  PLU=ON  MD2 OR MD 2
L2         3272 SEA FILE=WPIX ABB=ON  PLU=ON  ENDOTOXIN# OR ENDO (2W) TOXIN#
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L7          355 SEA FILE=WPIX ABB=ON  PLU=ON  WEISS J?/AU
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L9            1 SEA FILE=WPIX ABB=ON  PLU=ON  TEGHANEMT A?/AU
L10         316 SEA FILE=WPIX ABB=ON  PLU=ON  SUBRAMANIAN R?/AU
L11         670 SEA FILE=WPIX ABB=ON  PLU=ON  (L7 OR L8 OR L9 OR L10)
L12           1 SEA FILE=WPIX ABB=ON  PLU=ON  L11 AND L1
L13           2 SEA FILE=WPIX ABB=ON  PLU=ON  L11 AND L2
L14           2 SEA FILE=WPIX ABB=ON  PLU=ON  L12 OR L13
L15           1 SEA FILE=WPIX ABB=ON  PLU=ON  L14 NOT L3


=> d .wp l6 1-6;d bib ab l15

L6   ANSWER 1 OF 6  WPIX  COPYRIGHT 2006 THE THOMSON CORP on STN
AN   2006-253953 [26]   WPIX
DNC  C2006-082768
TI   New soluble Toll-like receptor 4 protein, useful as a therapeutic agent
     for treating endotoxin-induced inflammation.
DC   B04 D16
IN   HYAKUSHIMA, N; KUROKI, Y; MITSUZAWA, H
PA   (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY
CYC  112
PI   WO 2006033481   A1 20060330 (200626)* JA   78
```

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
KE LS LT LU LV MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ
UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KM KP KR KZ LC LK LR LS LT LU LV LY MA MD MG MK MN MW MX MZ NA NG
NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR
TT TZ UA UG US UZ VC VN YU ZA ZM ZW

ADT WO 2006033481 A1 WO 2005-JP18207 20050922
PRAI JP 2004-277421 20040924
AB WO2006033481 A UPAB: 20060421
NOVELTY - A soluble Toll-like receptor 4 protein (I) (TLR4), comprising an amino acid sequence having a fully defined 608 amino acid (SEQ ID No: 1) sequence, given in the specification, or an amino acid sequence that is substantially the same as SEQ ID Number 1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) DNA (II) encoding (I);
(2) recombinant vector (III) comprising (II);
(3) non-human host cell (IV) transformed with (III);
(4) preparing (I); and
(5) therapeutic agent (A1) comprising (I).

ACTIVITY - Antiinflammatory. In vivo analysis of soluble Toll-like receptor 4 protein (sTLR4) and **MD-2** in suppressing inflammation induced by **endotoxin** was carried out as follows. A female BALB/C mouse was anesthetized by ketamine HCl and xylazine hydrochloride. The mice injected with sTLR4 and **MD-2** protein were taken as a test group, and mice injected with sTLR2 was taken as control group. Lipopolysaccharide (LPS) (1 mu g) was dripped in trachea. After 16 hours, 1 ml of Hank's solution was used to wash bronchus alveolus. The concentration of tumor necrosis factor alpha in bronchus alveolus was measured. Results showed that inflammation induced by **endotoxin** was significantly reduced in test group.

MECHANISM OF ACTION - None given.

USE - (I) Or (A1) is useful for treating **endotoxin** induced inflammation (claimed), inflammation induced by nuclear factor kappa B activation, and interleukin-8 secretion.

DESCRIPTION OF DRAWING(S) - The figure is a graph representing the inflammatory cytokine of lung decreased by administration of soluble Toll-like receptor 4 protein and **MD-2.**

Dwg.9/9

TECH UPTX: 20060421
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: (I) Is prepared by culturing (IV), and expressing (I) (claimed).
Preferred Protein: (I) Comprises an amino acid sequence having SEQ ID No: 1, in which one or more amino acids are substituted, inserted, deleted or added. (I) Comprises SEQ ID No: 1.
Preferred Host Cell: (IV) Is of insect cell origin.
Preferred Agent: (A1) Is an antiinflammatory agent for treatment of **endotoxin**-induced inflammation.

L6 ANSWER 2 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2006-212808 [22] WPIX
DNC C2006-070123
TI New mutant or truncated **MD-2** polypeptide capable of specifically binding to lipopolysaccharide but having decreased binding affinity to toll-like receptor-4, for treating e.g. sepsis.
DC B04 D16
IN KIRKLAND, T N; VIRIYAKOSOL, S
PA (REGC) UNIV CALIFORNIA

CYC 111
PI WO 2006025995 A2 20060309 (200622)* EN 58
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
KE LS LT LU LV MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ
UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI
NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT
TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT WO 2006025995 A2 WO 2005-US26771 20050727
PRAI US 2005-681097P 20050513; US 2004-591805P 20040727
AB WO2006025995 A UPAB: 20060331
NOVELTY - A mutant or truncated **MD-2** polypeptide (P1), where (P1) specifically binds lipopolysaccharide (LPS) but cannot bind or has decreased binding affinity to toll-like receptor-4 (TLR4), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a chimeric protein (P2) comprising a first domain having an **MD-2** polypeptide and at least a second domain;

(2) a pharmaceutical composition (PC) comprising (P1) or (P2) and a excipient;

(3) a parenteral formulation (I) comprising (P1) or (P2);

(4) an enteral formulation (II) comprising (P1) or (P2);

(5) decreasing (M1) the amount of **endotoxin** in a biological fluid, comprising providing a composition or formulation comprising (P1) or (P2), and administering the composition or formulation to a subject in need of it, thus decreasing the amount of **endotoxin** in the biological fluid;

(6) an isolated or recombinant nucleic acid (N1) encoding (P1) or (P2);

(7) a vector or expression cassette (V1) comprising (N1);

(8) a host cell (H1) comprising (V1);

(9) a non-human transgenic animal comprising (V1) or (N1);

(10) a composition (C1) for transfecting nucleic acids into a cell, comprising (P1), and/or (P2), LPS and a nucleic acid; and

(11) transfecting (M2) a cell with nucleic acid, comprising providing a nucleic acid-comprising (C1), and contacting the cell with (C1) under conditions where (C1) is internalized into the cell.

ACTIVITY - Antibacterial; Immunomodulatory; Antiinflammatory; Respiratory-Gen.; Antiasthmatic; Virucide; Antiparasitic; Fungicide; Vulnerary; Antibacterial. No supporting data is given.

MECHANISM OF ACTION - Decreases **endotoxin** amount (claimed).

USE - (P1), (P2), (I) Or (II) is useful for decreasing the amount of **endotoxin** in a biological fluid, where the biological fluid comprises blood, serum or cerebrospinal fluid (CSF). PC Is useful for treating or ameliorating sepsis, which involves providing PC, and administering PC to a subject in need of it, thus treating the sepsis. PC Is useful for treating or ameliorating a condition comprising an LPS-induced disease, infection or pathology, which involves administering PC, where the LPS-induced disease or pathology is chosen from **endotoxin**-induced septic shock, **endotoxin**-induced toxic shock, sepsis, sever sepsis, septic shock caused by Gram-negative bacteria, bacterial infections, shock inflammatory diseases, graft versus host disease, autoimmune diseases, acute respiratory distress syndrome, granulomatous diseases, chronic infections, transplant rejection, acute respiratory asthma, viral infections, parasitic infections, fungal infections and trauma (all claimed). (P1) Is useful as a bacteria-targeting agent to treat sepsis caused by infectious diseases, such as bacterial and fungal diseases. (P1) Or (P2) is useful for

increasing clearance of LPS from circulation.

DESCRIPTION OF DRAWING(S) - The figure is a graph representing the effect of cell surface level of **MD-2** polypeptide on lipopolysaccharide activation.

Dwg.1/10

TECH UPTX: 20060331

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Polypeptide: (P1) Is a variant of the human **MD-2** having a fully defined 160 amino acid (SEQ ID No: 1) sequence, given in the specification, or a mature form of it lacking a signal sequence. (P1) Has any one of a fully defined 144 amino acid (SEQ ID No: 37, 38 or 39) sequence, given in the specification, or an amino acid sequence where one, several or all of the alanines in SEQ ID No: 1 or one, several or all of the alanines in a polyA domain of SEQ ID No: 37, 38 or 39 are replaced with a valine, leucine, an isoleucine or another aliphatic amino acid, or glycine, or equivalents, or their combination. (P1) Comprises a naturally secreted form of human **MD-2**, and is glycosylated. (P1) Comprises a dimeric **MD-2**, and an amino terminal fragment of the **MD-2** polypeptide. (P1) Comprises a peptidomimetic or synthetic protein. (P2) Comprises a recombinant fusion protein. The second domain comprises an opsinizing agent, where the opsinizing agent comprises an antibody Fc domain or an antibody that binds to an Fc receptor. (P2) Comprises two or more antibody Fc domains, or two or more **MD-2** polypeptides. The opsinizing agent comprises a human opsinizing agent. The **MD-2** polypeptide comprises a mature **MD-2** lacking a signal sequence, and a human **MD-2** polypeptide. The **MD-2** polypeptide comprises a mutant or truncated **MD-2** polypeptide, where the mutant or truncated polypeptide specifically binds LPS and binds TLR4 with an affinity less than that of wild type TLR4. The mutant or truncated polypeptide does not bind wild type TLR4. The **MD-2** variant has increased affinity for LPS, or TLR4 or CD14. The **MD-2** polypeptide comprises a mutation or truncation of a human **MD-2** polypeptide. (P2) Is soluble in aqueous media or the **MD-2** polypeptide comprises a naturally secreted form of human **MD-2**. (P2) Is glycosylated, and the **MD-2** polypeptide comprises a dimeric **MD-2**. The **MD-2** polypeptide comprises an amino terminal fragment of the **MD-2** polypeptide. The second domain comprises an Fc domain, a protein C, an antibacterial or bacteriostatic peptide or protein, antibiotic, a cytokine, an immunoregulatory agent, anti-inflammatory agent, a complement activating agent, carbohydrate-binding domain or their combination. The protein C is a human activated protein C. The complement activating agent comprises a collagen-like domain, fibrinogen-like domain or ficolin. (P2) Is a recombinant protein, and comprises a peptidomimetic or synthetic protein. The first domain is joined to the second domain by a chemical linking agent.

Preferred Host Cell: (H1) Is a bacterial cell, mammalian cell, fungal cell, an insect cell, a yeast cell or plant cell.

Preferred Composition: In (C1), the nucleic acid comprises:

(a) naked DNA or RNA, and optionally the naked DNA is operably linked to a promoter; or

(b) plasmid DNA, an expression cassette or expression vector.

The LPS comprises a bacterial LPS. (C1) Further comprises a lipopolysaccharide-binding protein (LBP) and/or a CD14 polypeptide.

Preferred Method: In (M2), the transfecting is an in vivo transfection or in vitro transfection. The cell is a bacterial cell or mammalian cell, where optionally the mammalian cell is a human cell.

L6 ANSWER 3 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2005-372350 [38] WPIX
DNN N2005-301093 DNC C2005-115401
TI New anti-TLR4-**MD-2** monoclonal antibody not exerting effect of B-cell proliferation inhibition and TNF production inhibition in macrophages, through in vitro lipopolysaccharide stimulation, for treating **endotoxin** shock.
DC B04 D16 S03
IN MIYAKE, K; TAKAMURA, S
PA (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY
CYC 108
PI WO 2005047330 A1 20050526 (200538)* JA 30
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT WO 2005047330 A1 WO 2004-JP14194 20040928
PRAI JP 2003-387173 20031117
AB WO2005047330 A UPAB: 20050616
NOVELTY - A monoclonal antibody (I) capable of specifically recognizing a TLR4-**MD-2** composite and not exerting effect of B-cell proliferation inhibition and TNF production inhibition in macrophages, through in vitro lipopolysaccharide (LPS) stimulation, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an anti-mouse TLR4-**MD-2** monoclonal antibody Sa 15-21 capable recognizing an antigenic determinant of mouse TLR4 in a mouse TLR4-**MD-2** composite, in the N-terminal end;

(2) an anti-human TLR4 monoclonal antibody TF904 capable of specifically recognizing the antigenic determinant of human TLR4, in the N-terminal end;

(3) a hybridoma capable of producing an anti-human TLR4 monoclonal antibody TF904 which specifically recognizes an antigenic determinant of human TLR4 (FER ABP-10118), in the N-terminal end;

(4) a therapeutic agent (A1) of **endotoxin** shock comprising (I); and

(5) screening an agent capable of promoting **endotoxin** shock inhibitory effect or substance inhibiting **endotoxin** shock, comprising administering anti-TLR4-**MD-2** mouse monoclonal antibody Sa 15-21 capable of recognizing TLR4-**MD-2** composite and a test substance, and evaluating the grade of **endotoxin** shock in a mouse, before and after the **endotoxin** shock.

ACTIVITY - Antibacterial; Immunosuppressive. No supporting data is given.

MECHANISM OF ACTION - TRL4-**MD-2** composite antagonist.

USE - (I) Is useful for treating or preventing **endotoxin** shock (claimed).

ADVANTAGE - (I) Effectively treats **endotoxin** shock.

Dwg.0/8
TECH UPTX: 20050616
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Antibody: (I) Enhances TNF production to **endotoxin** shock. (I) Recognizes an antigenic determinant of TLR4 in a TLR4-**MD-2** composite, in the N-terminal end.

L6 ANSWER 4 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2005-354786 [36] WPIX
DNC C2005-109712
TI New purified complexes comprising **endotoxin** bound to **MD-2**, useful for promoting innate immune response, as immunological adjuvants, or for treating conditions associated with **endotoxin**-mediated cell activation, e.g. sepsis.
DC B04 D16
IN GIOANNINI, T L; SUBRAMANIAN, R; TEGHANEMT, A; WEISS, J P
PA (GIOA-I) GIOANNINI T L; (SUBR-I) SUBRAMANIAN R; (TEGH-I) TEGHANEMT A; (WEIS-I) WEISS J P; (IOWA) UNIV IOWA RES FOUND
CYC 108
PI US 2005106179 A1 20050519 (200536)* 34
WO 2005049067 A1 20050602 (200536) EN
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT US 2005106179 A1 US 2003-715876 20031117; WO 2005049067 A1 WO 2004-US38375 20041117
PRAI US 2003-715876 20031117
AB US2005106179 A UPAB: 20050608
NOVELTY - A purified complex comprising **endotoxin** bound to **MD-2**, is new.
ACTIVITY - Antibacterial; Immunosuppressive; Hepatotropic; Gastrointestinal-Gen.; Antiinflammatory; CNS-Gen.; Respiratory-Gen.; Antiasthmatic; Cytostatic.
No biological data given.
MECHANISM OF ACTION - Gene therapy; Vaccine.
USE - The complex is useful for decreasing undesirable **endotoxin**-mediated inflammation, or for promoting innate immune response and as immunological adjuvants. The complex and methods may be used for treating conditions associated with **endotoxin**-mediated cell activation, such as sepsis, liver disease, inflammatory bowel disease, cystic fibrosis, asthma, autoimmune diseases, cancer or bacterial infections.
Dwg.0/14
TECH UPTX: 20050608
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Complex: The **endotoxin** is a wild-type **endotoxin** or a gram-negative bacterial **endotoxin**. The gram-negative bacterium is a Neisseria, Escherichia, Pseudomonas, Hemophilus, Salmonella or Francisella bacterium. The bacterium is preferably Neisseria meningitidis, Escherichia coli, Pseudomonas aeruginosa, Hemophilus influenzae, Salmonella typhimurium or Francisella tularensis. The complex has a molecular weight of about 25000. It consists essentially of one molecule of **endotoxin** bound to one molecule of **MD-2**. The complex is soluble in water.
It binds to Toll-like receptor 4 (TLR4) and produces TLR4-dependent activation of cells. The complex produces a half maximal TLR4-dependent activation of cells at a concentration of less than 1 nM of the complex, particularly about 30 pM or less of the complex. The **endotoxin** is hexa-acylated. It is an under-acylated **endotoxin**, a tetra-acylated **endotoxin** or a penta-acylated **endotoxin**. The complex produces less TLR4-dependent activation of cells as compared to a complex comprising an **endotoxin** that is hexa-acylated. A composition comprises the complex and a pharmaceutical carrier.

L6 ANSWER 5 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2004-143114 [14] WPIX
DNN N2004-114058 DNC C2004-057716
TI Extracorporeal adsorption agent for removing harmful substances that induce sepsis, by treating blood obtained from mammal by passing blood through adsorption column assembly at flow rate that fluidized bed of particles is formed.
DC B04 S03
IN HEEGAARD, P M H; LIHME, A O F
PA (UPFR-N) UPFRONT CHROMATOGRAPHY AS
CYC 105
PI WO 2004008138 A2 20040122 (200414)* EN 56
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
AU 2003242509 A1 20040202 (200450)
EP 1521624 A2 20050413 (200525) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
JP 2005532130 W 20051027 (200571) 48
US 2005249724 A1 20051110 (200574)
AU 2003242509 A8 20051103 (200629)
ADT WO 2004008138 A2 WO 2003-DK483 20030709; AU 2003242509 A1 AU 2003-242509 20030709; EP 1521624 A2 EP 2003-763618 20030709, WO 2003-DK483 20030709; JP 2005532130 W WO 2003-DK483 20030709, JP 2004-520339 20030709; US 2005249724 A1 WO 2003-DK483 20030709, US 2005-520685 20050527; AU 2003242509 A8 AU 2003-242509 20030709
FDT AU 2003242509 A1 Based on WO 2004008138; EP 1521624 A2 Based on WO 2004008138; JP 2005532130 W Based on WO 2004008138; AU 2003242509 A8 Based on WO 2004008138
PRAI DK 2002-1091 20020711
AB WO2004008138 A UPAB: 20040226
NOVELTY - Extracorporeal adsorption agent (M1), for removing harmful substances responsible of inducing sepsis caused by gram-negative or gram positive bacteria in a mammal, involves treating blood obtained from the mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed.

DETAILED DESCRIPTION - Extracorporeal adsorption (M1), for removing harmful substances responsible of inducing sepsis caused by gram-negative or gram positive bacteria in a mammal, the extracorporeal adsorption being effected by an adsorption column assembly, where the adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of the particles being at the most 80% of the volume of the column, the particles being characterized by carrying an affinity specific molecule with a specific affinity for the LPS portion of the gram-negative bacteria, gram-positive bacteria or harmful substances derived from the gram-positive bacteria, involves treating blood obtained from the mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed.

ACTIVITY - Antibacterial; Immunosuppressive.

MECHANISM OF ACTION - Removing harmful substances responsible of inducing sepsis.

The use of extracorporeal adsorption for the treatment of **endotoxin**-challenged cows was as follows. The cows weighing

500-800 kg were challenged by intravenous injection of 1000 ng lipopolysaccharide (LPS)/kg body weight. After the injection of LPS, the cow was connected to a venous-venous extracorporeal adsorption circuit, comprising a stabilized fluidized bed of polymyxin B-coated particles connected through a switch, the switch being activated by a continuous monitoring device, detecting changes in the serum concentration of haptoglobin in the blood. Clinical parameters, including rectal temperature, heat rate, respiratory frequency, and acute phase protein responses was measured up to one week after the challenge and compared between cows treated by the described extracorporeal method and in treated cows. Results showed that LPS-challenged cows treated by stand-by extracorporeal adsorption of the animal's blood in a continuous process through a stabilized fluidized bed of polymyxin B-coated particles present with significantly less, significantly less severe and significantly more short-lived clinical signs than comparable, non-treated cows.

USE - (M1) is useful for treating (M2) sepsis caused by gram-negative or gram-positive bacteria in mammal e.g., human being, which involves obtaining blood from the mammal, treating the obtained blood by passing the blood through the adsorption column assembly at such as flow rate that a fluidized bed of the particles is formed, and reinfusing the treated blood into the same mammal. The flow rate of the blood through the column assembly is such that expansion ratio of the fluidized bed is at least 1.3, such as at least 1.5. (M2) further involves injecting the substance into the blood stream of the mammal (claimed).

DESCRIPTION OF DRAWING(S) - The figure shows the principle of continuous extracorporeal adsorption.

Dwg.3/7

TECH UPTX: 20040226

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In (M1), the treated blood is capable of being reinfused into the same mammal. The adsorption column assembly is adapted for fluidized bed adsorption, in particular stabilized fluidized bed adsorption. The particles have a density of at least 1.3 g/ml and a mean diameter is 5-1000 microns, such as density of at least 1.5 g/ml and a mean diameter is 5-300 microns, preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 microns, and most preferably a density of more than 2.5 g/ml and a mean diameter is 5-75 microns. The affinity specific molecule is chosen from immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics and lectins. The affinity specific molecules are chosen from immunoglobulins. Two or more different specific molecules are present on particles within the adsorption medium. The affinity specific molecule is polymyxin B. The affinity specific molecule is chosen from toll-like receptor, most preferably TLR4 or its binding fragments or its multimeric arrangements, CD14, **MD2**, TLR2 and LBP, and their combinations. The sedimented volume of the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column. The stabilized fluidized bed is placed in line with a switch capable of being activated when a blood substance reaches a pre-set value, the blood substance is monitored by a device, the device is placed in line with the blood circulation, the device sending the activating signal to the switch when the value is reached.

L6 ANSWER 6 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2004-027278 [03] WPIX
DNN N2004-021623 DNC C2004-009400
TI Transgenic non human animal with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide, comprises **MD-2** gene deficient chromosome which encodes toll-like receptor.

DC B04 D16 P14 S03
PA (KAGA-N) KAGAKU GIJUTSU SHINKO JIGYODAN
CYC 1
PI JP 2003319734 A 20031111 (200403)* 13
ADT JP 2003319734 A JP 2002-130964 20020502
PRAI JP 2002-130964 20020502
AB JP2003319734 A UPAB: 20040112
NOVELTY - Transgenic non-human animal (I) with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide (LPS), comprises **MD-2** gene deficient chromosome which encodes toll-like receptor 4 (TLR4).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) screening (M1) a of Gram-negative-bacterial membrane component responsive substance, involves introducing a test substance into (I), or introducing a test substance into (I) having **MD-2** gene of different animal; and

(2) diagnosing (M2) the response of different **MD-2** gene in non-human animal, involves transducing **MD-2** gene into (I) and inducing an **endotoxin** shock into (I).

USE - (I) is useful for screening of Gram-negative-bacterial membrane component responsive substance, or for diagnosing the response of different **MD-2** genes in non-human animal (claimed). (I) is useful for developing a medical agent which is used for further drug development.

ADVANTAGE - (I) enables to screen Gram-negative-bacterial membrane component responsive substance, or to diagnose the response of different **MD-2** gene in non-human animal.

DESCRIPTION OF DRAWING(S) - The figure shows the lipopolysaccharide expression of the macrophage or dendritic cells derived from the **MD-2** genetically engineered mouse. (Drawing includes non-English language text).

Dwg.3/5

TECH UPTX: 20040112
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Transgenic Animal: (I) is a mouse. The plasmid (targeting vector) which comprises a poly A signal and a marker gene, substitutes **MD-2** gene fragment in a mouse. The targeting vector comprises a neomycin resistant gene which is substituted for the first exon of **MD-2** gene and diphtheria **toxin** gene connected with the 3' terminal of the **MD-2** gene. The targeting vector is linearized and transduced into an embryonic stem cell which is deficient in **MD-2** gene. The above embryonic stem cell is injected into the blastocyst of a mouse, thus a chimera mouse is produced. The chimera mouse and the wild-type mouse where crossed to produce a heterozygote. **MD-2** knockout mouse is obtained by intercrossing the heterozygote mouse.
Preferred Method: In (M1), the test substance introduces nucleotide polymorphism of human **MD-2** gene.

L15 ANSWER 1 OF 1 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 1992-217016 [26] WPIX
CR 1989-068849 [09]; 2000-678663 [63]; 2001-637906 [61]
DNC C1992-098276
TI Antibacterial fragments of bactericidal-permeability-increasing protein - for treating Gram negative bacterial infections especially in immuno suppressed patients.

DC B04 D16
IN ELSBACH, P; **WEISS, J**
PA (UYNY) UNIV NEW YORK STATE
CYC 18
PI WO 9209621 A1 19920611 (199226)* EN 63
RW: AT BE CH DE DK ES FR GB GR IT LU MC NL SE
W: AU CA JP
AU 9191275 A 19920625 (199239)
EP 563222 A1 19931006 (199340) EN
R: DE FR GB
EP 563222 A4 19940608 (199531)
US 5576292 A 19961119 (199701) 32
EP 563222 B1 19980225 (199812) EN 31
R: DE FR GB
DE 69128968 E 19980402 (199819)
ADT WO 9209621 A1 WO 1991-US9033 19911203; AU 9191275 A AU 1991-91275 19911203, WO 1991-US9033 19911203; EP 563222 A1 WO 1991-US9033 19911203, EP 1992-902215 19911203; EP 563222 A4 EP 1992-902215 ; US 5576292 A CIP of US 1987-84335 19870811, CIP of US 1988-228035 19880805, CIP of US 1990-621473 19901203, Cont of US 1991-754204 19910826, US 1993-173968 19931223; EP 563222 B1 WO 1991-US9033 19911203, EP 1992-902215 19911203; DE 69128968 E DE 1991-628968 19911203, WO 1991-US9033 19911203, EP 1992-902215 19911203
FDT AU 9191275 A Based on WO 9209621; EP 563222 A1 Based on WO 9209621; EP 563222 B1 Based on WO 9209621; DE 69128968 E Based on EP 563222, Based on WO 9209621
PRAI US 1991-754204 19910826; US 1990-621473 19901203;
US 1987-84335 19870811; US 1988-228035 19880805;
US 1993-173968 19931223
AB WO 9209621 A UPAB: 20011217
Protein (I) comprises specified aminoacid sequence of amino acids 1-199. Purified, isolated protein comprises amino acids 1-203; neutralising bacterial lipopolysaccharides (LPS) comprises exposing the LPS to a neutralising amount of protein (II) comprising N-terminal fragment of bactericidal/permeability-increasing protein (BPI); and treating a mammal suffering from or at an increased risk of contracting a gram-negative bacterial infection comprises administering (II). The bacteria are killed, or growth is inhibited, or the LPS is neutralised to prevent or abate clinical symptoms associated with the LPS, etc.

USE/ADVANTAGE - Used to treat mammals with gram-negative bacterial infections. The effectiveness of treatment is increased by administering the peptides with other bactericidal agents. Peptides will provide structural information for the design of future generations of antimicrobial agents, and used as probes into the mol. organisation of the multifunctional holo-BPI prote
Dwg.0/10

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     (FILE 'CAPLUS' ENTERED AT 11:38:24 ON 03 JUL 2006)

L1         22495 SEA ABB=ON  PLU=ON  TOXIN#/OBI (L) ENDO/OBI OR ENDOTOXIN#/OBI
L2           274 SEA ABB=ON  PLU=ON  MD 2/OBI
L3            26 SEA ABB=ON  PLU=ON  L1 (L) L2
L4            31 SEA ABB=ON  PLU=ON  L1 AND L2
L5        986912 SEA ABB=ON  PLU=ON  CONJUGAT?/OBI OR COMPLEX?/OBI
L6             8 SEA ABB=ON  PLU=ON  L4 AND L5
                 D SCAN
L7            14 SEA ABB=ON  PLU=ON  ((ENDO TOXIN# OR ENDOTOXIN# ) (4A) (MD2 OR
                 MD 2))/AB
L8            11 SEA ABB=ON  PLU=ON  L7 NOT L6
L9            62 SEA ABB=ON  PLU=ON  L1 (L) (BOUND/OBI OR BOND?/OBI)
L10            1 SEA ABB=ON  PLU=ON  L9 AND L2
                 D SCAN
L11            0 SEA ABB=ON  PLU=ON  ((ENDO TOXIN# OR ENDOTOXIN# ) (S) (BOUND
                 OR BOND?) (S)  (MD2 OR MD 2))/AB
L12           20 SEA ABB=ON  PLU=ON  L10 OR L6 OR L8
L13           14 SEA ABB=ON  PLU=ON  L12 AND  L1 AND L2
L14          362 SEA ABB=ON  PLU=ON  WEISS J/AU
L15           53 SEA ABB=ON  PLU=ON  GIOANNINI T?/AU
L16            8 SEA ABB=ON  PLU=ON  TEGHANEMT A?/AU
L17          909 SEA ABB=ON  PLU=ON  SUBRAMANIAN R?/AU
L18         1324 SEA ABB=ON  PLU=ON  (L14 OR L15 OR L16 OR L17)
L19           13 SEA ABB=ON  PLU=ON  L18 AND (L1 OR L2)
L20            6 SEA ABB=ON  PLU=ON  L7 AND L18
L21            5 SEA ABB=ON  PLU=ON  L18 AND L1 AND L2
L22            7 SEA ABB=ON  PLU=ON  L20 OR L21


     FILE 'MEDLINE' ENTERED AT 12:01:35 ON 03 JUL 2006
L23          410 SEA ABB=ON  PLU=ON  MD2 OR MD 2
L24          152 SEA ABB=ON  PLU=ON  LYMPHOCYTE ANTIGEN 96/CT
L25          425 SEA ABB=ON  PLU=ON  L23 OR L24
                 E ENDOTOXINS/CT
                 E E3+ALL
L26        18668 SEA ABB=ON  PLU=ON  ENDOTOXINS/CT
L27           23 SEA ABB=ON  PLU=ON  L25 AND L26
L28       941913 SEA ABB=ON  PLU=ON  CONJUGAT? OR BOUND? OR BOND? OR COMPLEX?
L29           15 SEA ABB=ON  PLU=ON  L27 AND L28
L30        30867 SEA ABB=ON  PLU=ON  ENDOTOXIN?
L31            7 SEA ABB=ON  PLU=ON  L25 (S) L28 (S) L30
                 E WEISS J?/AU
L32           19 SEA ABB=ON  PLU=ON  ("WEISS JERROLD"/AU OR "WEISS JERROLD
                 P"/AU)
                 E GIOANNINI T/AU
L33           44 SEA ABB=ON  PLU=ON  ("GIOANNINI T"/AU OR "GIOANNINI T L"/AU OR
                 "GIOANNINI THERESA L"/AU OR "GIOANNINI THERESA LEE"/AU)
                 E TEGHANEMT A/AU
L34            7 SEA ABB=ON  PLU=ON  ("TEGHANEMT A"/AU OR "TEGHANEMT ATHMANE"/AU
                 )
                 E SUBRAMANIAN R/AU
L35          122 SEA ABB=ON  PLU=ON  ("SUBRAMANIAN R"/AU OR "SUBRAMANIAN R
                 B"/AU OR "SUBRAMANIAN R M"/AU OR "SUBRAMANIAN R R"/AU OR
                 "SUBRAMANIAN R SHANKAR"/AU OR "SUBRAMANIAN R V"/AU OR "SUBRAMAN
                 IAN R VENKATA"/AU)
                 E SUBRAMANIAN RAMASWAMY/AU
L36            6 SEA ABB=ON  PLU=ON  "SUBRAMANIAN RAMASWAMY"/AU
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L37         185 SEA ABB=ON  PLU=ON  (L32 OR L33 OR L34 OR L35 OR L36)
L38           6 SEA ABB=ON  PLU=ON  L37 AND L25 AND L30
L39           3 SEA ABB=ON  PLU=ON  L38 NOT L31


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L40         534 SEA ABB=ON  PLU=ON  MD2 OR MD 2
                D HIT
L41       32504 SEA ABB=ON  PLU=ON  ENDO (2W) TOXIN# OR ENDOTOXIN#
L42          21 SEA ABB=ON  PLU=ON  L41 (S) L40
                D  HIT
L43      962150 SEA ABB=ON  PLU=ON  COMPLEX? OR BOUND? OR BOND? OR CONJUGAT?
L44          13 SEA ABB=ON  PLU=ON  L42 (L) L43
                E WEISS J/AU
L45         632 SEA ABB=ON  PLU=ON  "WEISS J"/AU OR ("WEISS J P"/AU OR "WEISS
                J PETER"/AU)
                E GIONNINI T/AU
                E GIOANNINI T/AU
L46          59 SEA ABB=ON  PLU=ON  ("GIOANNINI T"/AU OR "GIOANNINI T L"/AU OR
                "GIOANNINI THERESA"/AU OR "GIOANNINI THERESA L"/AU OR "GIOANNIN
                I THERESA LEE"/AU)
                E TREGHANEMT A/AU
                E TEGHANEMT  A/AU
L47          13 SEA ABB=ON  PLU=ON  ("TEGHANEMT A"/AU OR "TEGHANEMT ATHMANE"/AU
                )
                E SUBRAMANIAN R/AU
L48         179 SEA ABB=ON  PLU=ON  ("SUBRAMANIAN R"/AU OR "SUBRAMANIAN R
                B"/AU OR "SUBRAMANIAN R K"/AU OR "SUBRAMANIAN R R"/AU OR
                "SUBRAMANIAN R S"/AU OR "SUBRAMANIAN R SHANKAR"/AU OR "SUBRAMAN
                IAN R V"/AU)
                E SUBRAMANIAN RAMASWAMY/AU
L49          12 SEA ABB=ON  PLU=ON  "SUBRAMANIAN RAMASWAMY"/AU
L50         878 SEA ABB=ON  PLU=ON  (L45 OR L46 OR L47 OR L48 OR L49)
L51           5 SEA ABB=ON  PLU=ON  L50 AND L40 AND L41
L52           1 SEA ABB=ON  PLU=ON  L51 NOT L44

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L53          22 DUP REM L13 L31 L44 (12 DUPLICATES REMOVED)
                    ANSWERS '1-14' FROM FILE CAPLUS
                    ANSWERS '15-17' FROM FILE MEDLINE
                    ANSWERS '18-22' FROM FILE BIOSIS
L54           7 DUP REM L22 L39 L52 (4 DUPLICATES REMOVED)
                    ANSWERS '1-7' FROM FILE CAPLUS
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=> fil caplus medline biosis
FILE 'CAPLUS' ENTERED AT 12:18:30 ON 03 JUL 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'MEDLINE' ENTERED AT 12:18:30 ON 03 JUL 2006

FILE 'BIOSIS' ENTERED AT 12:18:30 ON 03 JUL 2006
Copyright (c) 2006 The Thomson Corporation

=> d que l53
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                 ENDOTOXIN#/OBI
L2           274 SEA FILE=CAPLUS ABB=ON  PLU=ON  MD 2/OBI
L4            31 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 AND L2
L5        986912 SEA FILE=CAPLUS ABB=ON  PLU=ON  CONJUGAT?/OBI OR COMPLEX?/OBI
L6             8 SEA FILE=CAPLUS ABB=ON  PLU=ON  L4 AND L5
L7            14 SEA FILE=CAPLUS ABB=ON  PLU=ON  ((ENDO TOXIN# OR ENDOTOXIN# )
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L8            11 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7 NOT L6
L9            62 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 (L) (BOUND/OBI OR BOND?/OBI)

L10            1 SEA FILE=CAPLUS ABB=ON  PLU=ON  L9 AND L2
L12           20 SEA FILE=CAPLUS ABB=ON  PLU=ON  L10 OR L6 OR L8
L13           14 SEA FILE=CAPLUS ABB=ON  PLU=ON  L12 AND  L1 AND L2
L23          410 SEA FILE=MEDLINE ABB=ON  PLU=ON  MD2 OR MD 2
L24          152 SEA FILE=MEDLINE ABB=ON  PLU=ON  LYMPHOCYTE ANTIGEN 96/CT
L25          425 SEA FILE=MEDLINE ABB=ON  PLU=ON  L23 OR L24
L28       941913 SEA FILE=MEDLINE ABB=ON  PLU=ON  CONJUGAT? OR BOUND? OR BOND?
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L30        30867 SEA FILE=MEDLINE ABB=ON  PLU=ON  ENDOTOXIN?
L31            7 SEA FILE=MEDLINE ABB=ON  PLU=ON  L25 (S) L28 (S) L30
L40          534 SEA FILE=BIOSIS ABB=ON  PLU=ON  MD2 OR MD 2
L41        32504 SEA FILE=BIOSIS ABB=ON  PLU=ON  ENDO (2W) TOXIN# OR ENDOTOXIN#

L42           21 SEA FILE=BIOSIS ABB=ON  PLU=ON  L41 (S) L40
L43       962150 SEA FILE=BIOSIS ABB=ON  PLU=ON  COMPLEX? OR BOUND? OR BOND? OR
                 CONJUGAT?
L44           13 SEA FILE=BIOSIS ABB=ON  PLU=ON  L42 (L) L43
L53           22 DUP REM L13 L31 L44 (12 DUPLICATES REMOVED)


=> d que l54
L1         22495 SEA FILE=CAPLUS ABB=ON  PLU=ON  TOXIN#/OBI (L) ENDO/OBI OR
                 ENDOTOXIN#/OBI
L2           274 SEA FILE=CAPLUS ABB=ON  PLU=ON  MD 2/OBI
L7            14 SEA FILE=CAPLUS ABB=ON  PLU=ON  ((ENDO TOXIN# OR ENDOTOXIN# )
                 (4A) (MD2 OR MD 2))/AB
L14          362 SEA FILE=CAPLUS ABB=ON  PLU=ON  WEISS J/AU
L15           53 SEA FILE=CAPLUS ABB=ON  PLU=ON  GIOANNINI T?/AU
L16            8 SEA FILE=CAPLUS ABB=ON  PLU=ON  TEGHANEMT A?/AU
L17          909 SEA FILE=CAPLUS ABB=ON  PLU=ON  SUBRAMANIAN R?/AU
L18         1324 SEA FILE=CAPLUS ABB=ON  PLU=ON  (L14 OR L15 OR L16 OR L17)
L20            6 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7 AND L18
L21            5 SEA FILE=CAPLUS ABB=ON  PLU=ON  L18 AND L1 AND L2
L22            7 SEA FILE=CAPLUS ABB=ON  PLU=ON  L20 OR L21
L23          410 SEA FILE=MEDLINE ABB=ON  PLU=ON  MD2 OR MD 2
L24          152 SEA FILE=MEDLINE ABB=ON  PLU=ON  LYMPHOCYTE ANTIGEN 96/CT
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L25         425 SEA FILE=MEDLINE ABB=ON  PLU=ON  L23 OR L24
L28      941913 SEA FILE=MEDLINE ABB=ON  PLU=ON  CONJUGAT? OR BOUND? OR BOND?
                OR COMPLEX?
L30       30867 SEA FILE=MEDLINE ABB=ON  PLU=ON  ENDOTOXIN?
L31           7 SEA FILE=MEDLINE ABB=ON  PLU=ON  L25 (S) L28 (S) L30
L32          19 SEA FILE=MEDLINE ABB=ON  PLU=ON  ("WEISS JERROLD"/AU OR "WEISS
                JERROLD P"/AU)
L33          44 SEA FILE=MEDLINE ABB=ON  PLU=ON  ("GIOANNINI T"/AU OR "GIOANNIN
                I T L"/AU OR "GIOANNINI THERESA L"/AU OR "GIOANNINI THERESA
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L34           7 SEA FILE=MEDLINE ABB=ON  PLU=ON  ("TEGHANEMT A"/AU OR "TEGHANEM
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L35         122 SEA FILE=MEDLINE ABB=ON  PLU=ON  ("SUBRAMANIAN R"/AU OR
                "SUBRAMANIAN R B"/AU OR "SUBRAMANIAN R M"/AU OR "SUBRAMANIAN R
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L36           6 SEA FILE=MEDLINE ABB=ON  PLU=ON  "SUBRAMANIAN RAMASWAMY"/AU
L37         185 SEA FILE=MEDLINE ABB=ON  PLU=ON  (L32 OR L33 OR L34 OR L35 OR
                L36)
L38           6 SEA FILE=MEDLINE ABB=ON  PLU=ON  L37 AND L25 AND L30
L39           3 SEA FILE=MEDLINE ABB=ON  PLU=ON  L38 NOT L31
L40         534 SEA FILE=BIOSIS ABB=ON  PLU=ON  MD2 OR MD 2
L41       32504 SEA FILE=BIOSIS ABB=ON  PLU=ON  ENDO (2W) TOXIN# OR ENDOTOXIN#

L42          21 SEA FILE=BIOSIS ABB=ON  PLU=ON  L41 (S) L40
L43      962150 SEA FILE=BIOSIS ABB=ON  PLU=ON  COMPLEX? OR BOUND? OR BOND? OR
                CONJUGAT?
L44          13 SEA FILE=BIOSIS ABB=ON  PLU=ON  L42 (L) L43
L45         632 SEA FILE=BIOSIS ABB=ON  PLU=ON  "WEISS J"/AU OR ("WEISS J
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L46          59 SEA FILE=BIOSIS ABB=ON  PLU=ON  ("GIOANNINI T"/AU OR "GIOANNINI
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                OR "GIOANNINI THERESA LEE"/AU)
L47          13 SEA FILE=BIOSIS ABB=ON  PLU=ON  ("TEGHANEMT A"/AU OR "TEGHANEMT
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L48         179 SEA FILE=BIOSIS ABB=ON  PLU=ON  ("SUBRAMANIAN R"/AU OR
                "SUBRAMANIAN R B"/AU OR "SUBRAMANIAN R K"/AU OR "SUBRAMANIAN R
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L49          12 SEA FILE=BIOSIS ABB=ON  PLU=ON  "SUBRAMANIAN RAMASWAMY"/AU
L50         878 SEA FILE=BIOSIS ABB=ON  PLU=ON  (L45 OR L46 OR L47 OR L48 OR
                L49)
L51           5 SEA FILE=BIOSIS ABB=ON  PLU=ON  L50 AND L40 AND L41
L52           1 SEA FILE=BIOSIS ABB=ON  PLU=ON  L51 NOT L44
L54           7 DUP REM L22 L39 L52 (4 DUPLICATES REMOVED)


=> d .ca l53 1-14;d ibib ab ct l53 15-22;d ibib ab ct l54 1-7

L53  ANSWER 1 OF 22  CAPLUS  COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER:         2005:1190853  CAPLUS
DOCUMENT NUMBER:          144:86458
TITLE:                    Pharmacological Inhibition of Endotoxin
                          Responses Is Achieved by Targeting the TLR4
                          Coreceptor, MD-2
AUTHOR(S):                Visintin, Alberto; Halmen, Kristen A.; Latz, Eicke;
                          Monks, Brian G.; Golenbock, Douglas T.
CORPORATE SOURCE:         Division of Infectious Diseases and Immunology,
                          University of Massachusetts Medical School, Worcester,
                          MA, 01655, USA
```

SOURCE: Journal of Immunology (2005), 175(10), 6465-6472
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 09 Nov 2005

AB The detection of Gram-neg. LPS depends upon the proper function of the TLR4-MD-2 receptor complex in immune cells. TLR4 is the signal transduction component of the LPS receptor, whereas **MD-2** is the **endotoxin**-binding unit. MD-2 appears to activate TLR4 when bound to TLR4 and ligated by LPS. Only the monomeric form of MD-2 was found to bind LPS and only monomeric MD-2 interacts with TLR4. Monomeric MD-2 binds TLR4 with an apparent Kd of 12 nM; this binding avidity was unaltered in the presence of endotoxin. E5564, an LPS antagonist, appears to inhibit cellular activation by competitively preventing the binding of LPS to MD-2. Depletion of endogenous soluble MD-2 from human serum, with an immobilized TLR4 fusion protein, abrogated TLR4-mediated LPS responses. By determining the concentration of added-back MD-2 that restored normal LPS responsiveness, the concentration of MD-2 was estimated to be .apprx.50 nM. Similarly, purified TLR4-Fc fusion protein, when added to the supernatants of TLR4-expressing cells in culture, inhibited the interaction of MD-2 with TLR4, thus preventing LPS stimulation. The ability to inhibit the effects of LPS as a result of the binding of TLR4-Fc or E5564 to MD-2 highlights MD-2 as the logical target for drug therapies designed to pharmacol. intervene against endotoxin-induced disease.

CC 15-10 (Immunochemistry)
Section cross-reference(s): 1, 13

ST **endotoxin** interaction TLR4 receptor MD2 protein

IT Drug targets
(**MD-2** protein)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; monomeric **MD-2** interacts with **endotoxin** or TLR4 receptor)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); monomeric **MD-2** interacts with **endotoxin** or TLR4 receptor)

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; monomeric **MD-2** interacts with **endotoxin** or TLR4 receptor)

IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**; monomeric **MD-2** interacts with **endotoxin** or TLR4 receptor)

IT Blood
(indexes; for **MD-2** protein)

IT Human
(monomeric **MD-2** interacts with **endotoxin** or TLR4 receptor)

IT Sepsis
(monomeric **MD-2** interacts with **endotoxin** or TLR4 receptor in relation to)

IT Stoichiometry
(of monomeric **MD-2** interaction with TLR4 receptor)

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS

L53 ANSWER 2 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2005:1016771 CAPLUS
DOCUMENT NUMBER: 143:304613
TITLE: Molecular Basis of Reduced Potency of Underacylated **Endotoxins**
AUTHOR(S): Teghanemt, Athmane; Zhang, DeSheng; Levis, Erika N.; Weiss, Jerrold P.; Gioannini, Theresa L.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA, 52241, USA
SOURCE: Journal of Immunology (2005), 175(7), 4669-4676
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 21 Sep 2005
AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxins depends on sequential endotoxin-protein and protein-protein interactions with LPS-binding protein, CD14, myeloid differentiation protein 2 (MD-2), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated endotoxins (i.e., tetra- or penta- vs. hexa-acylated) is determined by post-CD14 interactions. To better define the mol. basis of the differences in agonist potency of endotoxins differing in fatty acid acylation, the authors compared endotoxins (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB meningococcal strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of endotoxin:protein and endotoxin:cell interactions, the endotoxins were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric complexes with MD-2 in an LPS-binding protein- and CD14-dependent manner with similar efficiency. However, msbB LOS:MD-2 and acyloxyacylhydrolase-treated LOS:MD-2 were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:MD-2 and partially antagonized the action of wt LOS:MD-2. These findings suggest that underacylated endotoxins produce decreased TLR4-dependent cell activation by altering the interaction of the **endotoxin:MD-2** complex with TLR4 in a way that reduces receptor activation. Differences in potency among these endotoxin species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin:MD-2** complexes.
CC 15-10 (Immunochemistry)
Section cross-reference(s): 4, 10
ST acylation **endotoxin** TLR4 receptor activation
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
IT Toxins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**endotoxins**; structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL

(Biological study)
(lipooligosaccharides; structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
IT Structure-activity relationship
(of acylation variants of **endotoxins**)
IT Cell activation
(structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
IT 140936-27-2 864814-65-3 864814-66-4
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(structural features of **endotoxins** inducing TLR4 receptor-dependent cell activation)
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 3 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2005:489362 CAPLUS
DOCUMENT NUMBER: 143:95745
TITLE: Monomeric **endotoxin**:protein **complexes** are essential for TLR4-dependent cell activation
AUTHOR(S): Gioannini, T. L.; Teghanemt, A.; Zhang, De S.; Levis, E. N.; Weiss, J. P.
CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa and the Veterans' Administration Medical Center, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2005), 11(2), 117-123
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 09 Jun 2005
AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxin depends on sequential endotoxin-protein and protein-protein interactions with LBP, CD14, MD-2 and TLR4. LBP and CD14 combine, in an albumin-dependent fashion, to extract single endotoxin mols. from purified endotoxin aggregates (Eagg) or the bacterial outer membrane and form monomeric endotoxin:CD14 complexes that are the preferred presentation of endotoxin for transfer to MD-2. Endotoxin in endotoxin:CD14 is readily transferred to MD-2, again in an albumin-dependent manner, to form monomeric endotoxin:MD-2 complex. This monomeric endotoxin:protein complex (endotoxin:MD-2) activates TLR4 at picomolar concns., independently of albumin, and is, therefore, the apparent ligand in endotoxin-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated forms of meningococcal endotoxin (LOS) react similarly with LBP, CD14, and MD-2 to form endotoxin:MD-2 complexes. However, tetra- and penta-acylated LOS:MD-2 complexes are less potent TLR4 agonists than hexa-acylated LOS:MD-2. This is mirrored in the reduced activity of tetra-, penta- vs. hexa-acylated LOS aggregates (LOSagg) + LBP toward cells containing mCD14, MD-2, and TLR4. Therefore, changes in agonist potency of under-acylated meningococcal LOS are determined by differences in properties of monomeric endotoxin:MD-2.
CC 15-10 (Immunochemistry)
ST **endotoxin** protein **complex** TLR4 receptor cell

activation
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Blood vessel
(endothelium; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Toxins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**endotoxins**; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Glycolipids
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(lipooligosaccharides; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Cell activation
(monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(serum; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
IT Endothelium
(vascular; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 4 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 2004:292853 CAPLUS
DOCUMENT NUMBER: 140:401625
TITLE: Isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations
AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zhang, DeSheng; Coussens, Nathan P.; Dockstader, Wendie; Ramaswamy, S.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, and Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA, 52242, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(12), 4186-4191

CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 09 Apr 2004
AB Host proinflammatory responses to minute amts. of endotoxins derived from many Gram-neg. bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and MD-2. Optimal sensitivity to endotoxin requires an ordered series of endotoxin-protein and protein-protein interactions. At substoichiometric concns., LBP facilitates delivery of endotoxin aggregates to soluble CD14 (sCD14) to form monomeric endotoxin-sCD14 complexes. Subsequent interactions of endotoxin-sCD14 with TLR4 and/or MD-2 have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive endotoxin-MD-2 complex generated by treatment of endotoxin-sCD14 with recombinant MD-2. Efficient generation of this complex occurred at picomolar concns. of endotoxin and nanogram per mL doses of MD-2 and required presentation of endotoxin to MD-2 as a monomeric endotoxin-CD14 complex. TLR4-dependent delivery of endotoxin to human embryonic kidney (HEK) cells and cell activation at picomolar concns. of endotoxin occurred with the purified endotoxin-MD-2 complex, but not with purified endotoxin aggregates with or without LBP and/or sCD14. The presence of excess MD-2 inhibited delivery of endotoxin-MD-2 to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concns. of endotoxin occurs by sequential interaction and transfer of endotoxin to LBP, CD14, and MD-2 and simultaneous engagement of endotoxin and TLR4 by MD-2.
CC 4-5 (Toxicology)
ST **endotoxin** MD2 **complex** CD14 TLR4 LBP
IT Animal cell line
(HEK; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(LPS-LBP (lipopolysaccharide-containing lipopolysaccharide-binding protein); isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complexes** with **endotoxin**; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**complexes** with **endotoxin**; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT Toxins
RL: ADV (Adverse effect, including toxicity); BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**, **complexes** with CD14 and **MD-**

2; isolation of an **endotoxin-MD-2** **complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT Human
(isolation of an **endotoxin-MD-2** **complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 5 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 8

ACCESSION NUMBER: 2004:1074780 CAPLUS
DOCUMENT NUMBER: 142:238068
TITLE: **Endotoxin** recognition and signal transduction by the TLR4/MD2-**complex**
AUTHOR(S): Fitzgerald, Katherine A.; Rowe, Daniel C.; Golenbock, Douglas T.
CORPORATE SOURCE: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, 01605, USA
SOURCE: Microbes and Infection (2004), 6(15), 1361-1367
CODEN: MCINFS; ISSN: 1286-4579
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
ED Entered STN: 16 Dec 2004
AB A review. Bacterial lipopolysaccharides are recognized in mammals by a receptor complex composed of CD14, Toll-like receptor (TLR)-4, and MD-2. Transduction of signaling is achieved following the recruitment of a combination of four Toll-interleukin-1 resistance (TIR)-domain-containing adapter mols., which provide a structural platform enabling the recruitment and activation of downstream effectors essential for pathway-specific transcription factor activation and inflammatory gene expression.
CC 15-0 (Immunochemistry)
Section cross-reference(s): 14
ST review **endotoxin** lipopolysaccharide recognition signaling TLR4 MD2 **complex**
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; bacterial lipopolysaccharide/ **endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); bacterial lipopolysaccharide/ **endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial lipopolysaccharide/**endotoxin** recognition and signal transduction by CD14/TLR4/MD2 **complex**)
IT Inflammation
Signal transduction, biological
(bacterial lipopolysaccharide/**endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; bacterial lipopolysaccharide/**endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 6 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:451419 CAPLUS
DOCUMENT NUMBER: 142:476294
TITLE: **Endotoxin** shock medicine for TLR4-**MD**-**2** composite as target
INVENTOR(S): Miyake, Kensuke; Takamura, Sachiko
PATENT ASSIGNEE(S): Japan Science and Technology Agency, Japan
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005047330	A1	20050526	WO 2004-JP14194	20040928

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: JP 2003-387173 A 20031117
ED Entered STN: 27 May 2005
AB There are provided an anti-TLR4-MD-2 monoclonal antibody capable of specifically recognizing TLR4-**MD**-**2** composite; an **endotoxin** shock medicine for TLR4-**MD**-**2** composite as target, comprising the anti-TLR4-MD-2 monoclonal antibody; and a method of treating endotoxin shock medicine with the use of the anti-TLR4-MD-2 monoclonal antibody against TLR4-MD-2 composite as target. The anti-TLR4-MD-2 monoclonal antibody having an effect of suppressing endotoxin shock is produced by increasing the production of TNF against endotoxin shock, while not exerting an effect of B-cell proliferation inhibition and an effect of TNF production inhibition in macrophages, through in vitro LPS stimulation.
IC ICM C07K016-28
ICS A61K039-395; A61P039-02; G01N033-15; G01N033-50; C12P021-08
CC 1-12 (Pharmacology)
ST **endotoxin** shock monoclonal antibody anti tlrmd2
IT Cell proliferation
(B cell; **endotoxin** shock medicine for TLR4-**MD**-**2** composite as target)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); **endotoxin** shock medicine for TLR4-**MD**-**2** composite as target)
IT Macrophage
(**endotoxin** shock medicine for TLR4-**MD**-**2** composite as target)
IT Tumor necrosis factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(**endotoxin** shock medicine for TLR4-**MD-2** composite as target)

IT Toxins
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(**endotoxins**; **endotoxin** shock medicine for TLR4-**MD-2** composite as target)

IT Antibodies and Immunoglobulins
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(monoclonal, anti-TLR4-**MD-2**; **endotoxin** shock medicine for TLR4-**MD-2** composite as target)

IT B cell (lymphocyte)
(proliferation; **endotoxin** shock medicine for TLR4-**MD-2** composite as target)

IT Shock (circulatory collapse)
(septic; **endotoxin** shock medicine for TLR4-**MD-2** composite as target)

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 7 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:426231 CAPLUS
DOCUMENT NUMBER: 142:480799
TITLE: Preparation of **complexes** of **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**
INVENTOR(S): Weiss, Jerrold P.; Gioannini, Theresa L.; Teghanemt, Athamane; Subramanian, Ramaswamy
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 34 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005106179	A1	20050519	US 2003-715876	20031117
WO 2005049067	A1	20050602	WO 2004-US38375	20041117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-715876 A 20031117
ED Entered STN: 19 May 2005
AB The disclosed invention provides purified water soluble complexes of endotoxin and MD-2. The invention also provides a method for making the complexes of the invention and a method for isolating complexes of the invention. Also provided are the method of using the complexes of the invention, e.g. method to increase or inhibit TLR4 receptor-dependent activation of cells by endotoxin in vitro or in vivo. Methods using

complexes with mutant endotoxin are useful to decrease undesirable endotoxin-mediated inflammation. Methods using complexes with wild-type endotoxin are of use in promoting innate immunity and as immune adjuvants. The results of one example demonstrate that in primary cultures of human airway epithelia TLR4, but little or no MD-2 is expressed, so the cells are relatively unresponsive to added endotoxin. However, the cell responsiveness to endotoxin is markedly amplified by either the endogenous expression or exogenous addition of MD-2.

IC ICM A61K039-02
ICS C07K014-195
INCL 424235100; 530395000
CC 15-10 (Immunochemistry)
ST **endotoxin** MD2 **complex** TLR4 receptor cell activation
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(CD14 requirement in preparation of **complexes** of bacterial **endotoxin** and **MD-2**)
IT Animal cell line
(Hek 293; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Proteins
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**MD-2**, **endotoxin complexes**; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Immunostimulants
(adjuvants; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)
IT Glycolipids
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(bacterial; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Drug delivery systems
(carriers; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Toxins
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**endotoxins**, **MD-2 complexes**; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Toxins
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)

(**endotoxins**, acylated; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

IT Respiratory system
(epithelium; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT Immunity
(innate; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT Cell activation
Escherichia
Escherichia coli
Francisella
Francisella tularensis
Haemophilus
Haemophilus influenzae
Neisseria
Neisseria meningitidis
Pseudomonas
Pseudomonas aeruginosa
Salmonella
Salmonella typhimurium
(preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

IT Anti-inflammatory agents
Human
(preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT Epithelium
(respiratory tract; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT 852008-84-5 852009-59-7 852009-60-0 852009-61-1 852009-62-2
852009-63-3 852009-64-4 852009-65-5 852009-66-6 852009-67-7
852009-68-8 852009-69-9 852009-70-2 852009-71-3 852009-72-4
852009-73-5
RL: PRP (Properties)
(unclaimed nucleotide sequence; preparation of **complexes** of **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

L53 ANSWER 8 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:1089125 CAPLUS
DOCUMENT NUMBER: 142:175247
TITLE: Protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions
AUTHOR(S): Asai, Yasuyuki; Takaori, Kyoichi; Yamamoto, Tsuyoshi; Ogawa, Tomohiko
CORPORATE SOURCE: Department of Oral Microbiology, Asahi University School of Dentistry, Mizuho, Gifu, 501-0296, Japan

SOURCE: FEMS Immunology and Medical Microbiology (2005), 43(1), 91-98
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 20 Dec 2004

AB The protein-bound polysaccharide isolated from basidiomycetes (PSK) is a biol. response modifier capable of exhibiting various biol. activities, such as antitumor and antimicrobial effects. In the present study, the authors found that PSK suppressed interleukin (IL)-6 production in murine peritoneal macrophages stimulated with endotoxic lipopolysaccharide (LPS) and its synthetic lipid A (compound 506). Nitric oxide production and p38 mitogen-associated protein kinase phosphorylation induced in a murine macrophage cell line, J774-A1, by LPS and compound 506 were also inhibited by PSK. Further, PSK distinctly suppressed nuclear factor-κB activation in Ba/F3 cells expressing mouse Toll-like receptor 4 and MD-2, following stimulation with LPS and compound 506, however, not with Taxol. These PSK-induced inhibitory activities were caused by inhibition of the phys. assocns. of LPS with LPS-binding protein (LBP) and CD14. PSK also protected mice from LPS-induced lethality, presumably by down-regulating IL-6 and tumor necrosis factor-α concns. in serum. These findings indicate that PSK, which also has an ability to regulate LBP/CD14 functions, may be useful for clin. control of endotoxic sepsis.

CC 15-10 (Immunochemistry)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(NF-κB (nuclear factor of κ light chain gene enhancer in B-cells); protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipopolysaccharide-binding; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)

IT Peritoneum
(macrophage; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)

IT Macrophage
(peritoneal; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)

IT Basidiomycota
(protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking

lipopolysaccharide-binding protein and CD14 functions)
IT CD14 (antigen)
Interleukin 6
Tumor necrosis factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)
IT Polysaccharides, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein-**bound**; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)
IT 10102-43-9, Nitric oxide, biological studies 165245-96-5, p38 MAP kinase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions)
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 9 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:70134 CAPLUS
DOCUMENT NUMBER: 142:152686
TITLE: **Endotoxin** shock and TLR
AUTHOR(S): Takamura, Sachiko Akashi
CORPORATE SOURCE: Inst. Med. Sci., The Univ. Tokyo, Japan
SOURCE: Ensho to Men'eki (2004), Volume Date 2005, 13(1), 37-44
CODEN: ENMEFA; ISSN: 0918-8371
PUBLISHER: Sentan Igakusha
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
ED Entered STN: 27 Jan 2005
AB A review on roles of Toll-like receptor (TLR) in endotoxin shock, discussing recognition mechanism of **endotoxin** (lipopolysaccharide : LPS) by TLR4/**MD-2** complex, essential role of **MD-2** in LPS responsiveness and **endotoxin** shock, MD proteins regulating LPS recognition and signal transduction, and possible therapeutic application of monoclonal antibodies inhibiting TLR4/MD-2 functions.
CC 14-0 (Mammalian Pathological Biochemistry)
Section cross-reference(s): 15
ST review **endotoxin** shock TLR receptor lipopolysaccharide recognition
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; **endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin** shock)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR (Toll-like receptor); **endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin** shock)
IT Lipopolysaccharides
RL: ADV (Adverse effect, including toxicity); BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; **endotoxin** (lipopolysaccharide) recognition

mechanism by Toll-like receptor (TLR) in relation to **endotoxin** shock)

IT Molecular recognition
(**endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin** shock)

IT Toxins
RL: ADV (Adverse effect, including toxicity); BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**; **endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin** shock)

IT Shock (circulatory collapse)
(septic; **endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin** shock)

L53 ANSWER 10 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:70743 CAPLUS
DOCUMENT NUMBER: 140:355309
TITLE: **Endotoxin** recognition molecules, Toll-like receptor 4-**MD-2**
AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: The Institute of Medical Science, Department of Microbiology and Immunology, Division of Infectious Genetics, The University of Tokyo, 4-6-1 Shirokanedai, Tokyo, 108-8639, Japan
SOURCE: Seminars in Immunology (2004), 16(1), 11-16
CODEN: SEIME2; ISSN: 1044-5323
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
ED Entered STN: 29 Jan 2004
AB A review. Toll-like receptors (TLRs) are innate pathogen recognition mols. for microbial products. Lipopolysaccharide (LPS), a membrane constituent of Gram-neg. bacteria, is one of the most potent microbial products. LPS is recognized by TLR4 and MD-2. TLR4 is a transmembrane protein, the extracellular domain of which is composed of a protein motif called leucine-rich repeats (LRR). MD-2 is an extracellular mol. that is associated with the extracellular LRR of TLR4. MD-2 has a role in cell surface expression of TLR4 and interaction with LPS. TLR4-MD-2 contributes to containment of infections by Gram-neg. bacteria by activating immune responses.
CC 15-0 (Immunochemistry)
ST review **endotoxin** TLR4 receptor **complex** MD2 protein
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complexes**, with TLR-4; in immune recognition of bacterial **endotoxin**)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **complexes**, with **MD-2**; in immune recognition of bacterial **endotoxin**)

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; TLR-4 receptor/**MD-2** accessory protein in immune recognition of)

IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**; TLR-4 receptor/**MD-2** accessory protein in immune recognition of)

IT Immunity

(innate; TLR-4 receptor/**MD-2** accessory protein in recognition of bacterial **endotoxin**)
REFERENCE COUNT: 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 11 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:683145 CAPLUS
DOCUMENT NUMBER: 142:296120
TITLE: Molecular mechanism of **endotoxin** (LPS) recognition
AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: Institute of Medical Science, University of Tokyo, Japan
SOURCE: Endotokishin Kenkyu (2003), 6, 23-30
CODEN: EKNEBO
PUBLISHER: Igaku Tosho Shuppan K.K.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
ED Entered STN: 23 Aug 2004
AB A review. The topics discussed are (1) processing of lipopolysaccharide (LPS); (2) Toll-like receptor 4 (TLR4) in the recognition of LPS; (3) MD-2 binding to TLR4; (4) MD-2 required for cell surface expression of TLR4; (5) role of MD-2 in TLR4 recognition of LPS; and (6) RP105-MD-1 complex in B cell recognition of LPS.
CC 15-0 (Immunochemistry)
ST review Toll like receptor TLR4 MD2 **endotoxin** lipopolysaccharide
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complexes** with TLR4; Toll-like receptor 4-**MD-2 complex** in recognition of **endotoxin**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **complexes** with **MD-2**; Toll-like receptor 4-**MD-2 complex** in recognition of **endotoxin**)
IT Molecular association
Molecular recognition
(Toll-like receptor 4-**MD-2 complex** in recognition of **endotoxin**)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; Toll-like receptor 4-**MD-2 complex** in recognition of **endotoxin**)
IT Immunity
(innate; Toll-like receptor 4-**MD-2 complex** in recognition of **endotoxin**)

L53 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:588215 CAPLUS
DOCUMENT NUMBER: 138:70580
TITLE: TLR4-MD2 signaling pathway induced by **endotoxin**
AUTHOR(S): Li, Yongwang; Ma, Li; Mao, Baoling; Qian, Guisheng
CORPORATE SOURCE: Institute of Respiratory Disease, Xinqiao Hospital, the Third Military Medical University, Chungking, 400037, Peop. Rep. China
SOURCE: Zhongguo Yaolixue Tongbao (2002), 18(2), 121-125
CODEN: ZYTOE8; ISSN: 1001-1978
PUBLISHER: Anhui Yike Daxue Linchuan Yaoli Yanjiuso

DOCUMENT TYPE: Journal; General Review
LANGUAGE: Chinese
ED Entered STN: 08 Aug 2002
AB A review with 24 refs. on TLR4-MD2 (TLR4 = toll-like receptor-4) signaling pathway induced by endotoxin with subdivision headings: (1) survey on TLRs; (2) role of TLR4 and its accessory protein MD2 in signaling pathway; (3) basic composition of lipopolysaccharide (LPS) signaling pathway mediated by TLR4-MD2; Biol. significance of TLR4-MD2 signaling pathway deficiency; (5) expression and role of TLR4-MD2 in different tissues; and (6) conclusions.
CC 14-0 (Mammalian Pathological Biochemistry)
ST review TLR4 MD2 signaling pathway **endotoxin** inflammation
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complex** with TLR-4; TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(NF-κB (nuclear factor of κ light chain gene enhancer in B-cells); TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **complex** with **MD-2**; TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Inflammation
Signal transduction, biological
(TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Lipopolysaccharides
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(TLR4- MD2 signaling pathway induced by **endotoxin**)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Toxins
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(**endotoxins**; TLR4- MD2 signaling pathway induced by **endotoxin**)

L53 ANSWER 13 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:115771 CAPLUS
DOCUMENT NUMBER: 138:335921
TITLE: Role of glucosylation of **MD-2** in **endotoxin** signal transduction
AUTHOR(S): Onishi, Takahiro; Muroi, Masashi; Tanamoto, Kenichi
CORPORATE SOURCE: Division of Microorganisms, National Institute of Health Sciences, Japan
SOURCE: Endotokishin Kenkyu (2002), 5, 50-55
CODEN: EKNEBO
PUBLISHER: Igaku Tosho Shuppan K.K.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
ED Entered STN: 14 Feb 2003
AB A review discusses role of glucosylation of **MD-2** protein in signaling of **endotoxin** in monocytes, and involvement of TLR4 receptor.
CC 15-0 (Immunochemistry)
ST review glucosylation MD2 protein **endotoxin** signaling
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; role of glucosylation of **MD-2** in **endotoxin** signal transduction)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); role of glucosylation of **MD-2** in **endotoxin** signal transduction)
IT Glucosylation
Human
Macrophage
Signal transduction, biological
(role of glucosylation of **MD-2** in **endotoxin** signal transduction)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(role of glucosylation of **MD-2** in **endotoxin** signal transduction)

L53 ANSWER 14 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:491171 CAPLUS
DOCUMENT NUMBER: 136:149763
TITLE: Molecular genetic analysis of an **endotoxin** nonresponder mutant cell line: a point mutation in a conserved region of **MD-2** abolishes **endotoxin**-induced signaling
AUTHOR(S): Schromm, Andra B.; Lien, Egil; Henneke, Philipp; Chow, Jesse C.; Yoshimura, Atsutoshi; Heine, Holger; Latz, Eicke; Monks, Brian G.; Schwartz, David A.; Miyake, Kensuke; Golenbock, Douglas T.
CORPORATE SOURCE: Evans Biomedical Research Center, Boston University School of Medicine, Boston, MA, 02118, USA
SOURCE: Journal of Experimental Medicine (2001), 194(1), 79-88
CODEN: JEMEAV; ISSN: 0022-1007
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 08 Jul 2001
AB Somatic cell mutagenesis is a powerful tool for characterizing receptor systems. We reported previously two complementation groups of mutant cell lines derived from CD14-transfected Chinese hamster ovary-K1 fibroblasts defective in responses to bacterial endotoxin. Both classes of mutants expressed a normal gene product for Toll-like receptor (TLR)4, and fully responded to stimulation by tumor necrosis factor (TNF)-α or interleukin (IL)-1β. We identified the lesion in one of the complementation groups in the gene for MD-2, a putative TLR4 coreceptor. The nonresponder phenotype of this mutant was reversed by transfection with MD-2. Cloning of MD-2 from the nonresponder cell line revealed a point mutation in a highly conserved region resulting in a C95Y amino acid exchange. Both forms of MD-2 colocalized with TLR4 on the cell surface after transfection, but only the wild-type cDNA reverted the lipopolysaccharide (LPS) nonresponder phenotype. Furthermore, soluble MD-2, but not soluble MD-2C95Y, functioned to enable LPS responses in cells that expressed TLR4. Thus, MD-2 is a required component of the LPS signaling complex and can function as a soluble receptor for cells that do not otherwise express it. We hypothesize that MD-2 conformationally affects the extracellular domain of TLR4, perhaps resulting in a change in affinity for LPS or functioning as a portion of the true ligand for TLR4.
CC 15-10 (Immunochemistry)
ST MD2 Toll like receptor **endotoxin** signaling; lipopolysaccharide signaling MD2 TLR4
IT Signal transduction, biological
(LPS signaling, **MD-2** is required component of; point mutation in conserved region of **MD-2**

abolishes **endotoxin**-induced signaling)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(LPS, **MD-2** is required component of LPS signaling **complex**; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**MD-2**, TLR4 coreceptor; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **MD-2** colocalized with; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
IT Protein motifs
(conserved region, of **MD-2**, C95Y substitution in; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**, LPS; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
IT Gram-negative bacteria
(point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
IT Mutation
(point; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 15 OF 22 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2005211589 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15845500
TITLE: Differential induction of the toll-like receptor 4-MyD88-dependent and -independent signaling pathways by endotoxins.
AUTHOR: Zughaier Susu M; Zimmer Shanta M; Datta Anup; Carlson Russell W; Stephens David S
CORPORATE SOURCE: Division of Infectious Diseases, Emory University School of Medicine, VAMC (I-151), 1670 Clairmont Rd, Atlanta, GA 30033, USA.. szughai@emory.edu
CONTRACT NUMBER: R01 AI033517-10 (NIAID)
SOURCE: Infection and immunity, (2005 May) Vol. 73, No. 5, pp. 2940-50.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 23 Apr 2005
Last Updated on STN: 8 Jun 2005
Entered Medline: 7 Jun 2005

AB The biological response to **endotoxin** mediated through the Toll-like receptor 4 (TLR4)-**MD-2** receptor **complex** is directly related to lipid A structure or configuration. Endotoxin structure may also influence activation of the MyD88-dependent and -independent signaling pathways of TLR4. To address this possibility, human macrophage-like cell lines (THP-1, U937, and MM6) or murine macrophage RAW 264.7 cells were stimulated with picomolar concentrations of highly purified endotoxins. Harvested supernatants from previously stimulated cells were also used to stimulate RAW 264.7 or 23ScCr (TLR4-deficient) macrophages (i.e., indirect induction). Neisseria meningitidis lipooligosaccharide (LOS) was a potent direct inducer of the MyD88-dependent pathway molecules tumor necrosis factor alpha (TNF-alpha), interleukin-1beta (IL-1beta), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 3alpha (MIP-3alpha), and the MyD88-independent molecules beta interferon (IFN-beta), nitric oxide, and IFN-gamma-inducible protein 10 (IP-10). Escherichia coli 55:B5 and Vibrio cholerae lipopolysaccharides (LPSs) at the same pmole/ml lipid A concentrations induced comparable levels of TNF-alpha, IL-1beta, and MIP-3alpha, but significantly less IFN-beta, nitric oxide, and IP-10. In contrast, LPS from Salmonella enterica serovars Minnesota and Typhimurium induced amounts of IFN-beta, nitric oxide, and IP-10 similar to meningococcal LOS but much less TNF-alpha and MIP-3alpha in time course and dose-response experiments. No MyD88-dependent or -independent response to endotoxin was seen in TLR4-deficient cell lines (C3H/HeJ and 23ScCr) and response was restored in TLR4-MD-2-transfected human embryonic kidney 293 cells. Blocking the MyD88-dependent pathway by DNMyD88 resulted in significant reduction of TNF-alpha release but did not influence nitric oxide release. IFN-beta polyclonal antibody and IFN-alpha/beta receptor 1 antibody significantly reduced nitric oxide release. N. meningitidis endotoxin was a potent agonist of both the MyD88-dependent and -independent signaling pathways of the TLR4 receptor complex of human macrophages. E. coli 55:B5 and Vibrio cholerae LPS, at the same picomolar lipid A concentrations, selectively induced the MyD88-dependent pathway, while Salmonella LPS activated the MyD88-independent pathway.

CT Adaptor Proteins, Signal Transducing
Animals
*Antigens, Differentiation: ME, metabolism
Cell Line
Cytokines: ME, metabolism
*Endotoxins: CH, chemistry
*Endotoxins: PH, physiology
Gram-Negative Bacteria: IM, immunology
Gram-Negative Bacteria: ME, metabolism
Gram-Negative Bacteria: PY, pathogenicity
Humans
Lipid A: CH, chemistry
Lipid A: PD, pharmacology
Lipopolysaccharides: CH, chemistry
Macrophage Activation: DE, drug effects
*Macrophage Activation: IM, immunology
Macrophages: IM, immunology
Macrophages: ME, metabolism
*Membrane Glycoproteins: ME, metabolism
Mice
Mice, Inbred C3H
Nitric Oxide: ME, metabolism
*Receptors, Cell Surface: ME, metabolism
*Receptors, Immunologic: ME, metabolism
Research Support, N.I.H., Extramural

Research Support, U.S. Gov't, P.H.S.
*Signal Transduction
Toll-Like Receptor 4
Toll-Like Receptors

L53 ANSWER 16 OF 22 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2005303694 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15949133
TITLE: Detoxifying endotoxin: time, place and person.
AUTHOR: Munford Robert S
CORPORATE SOURCE: Molecular Host Defense Laboratory, Departments of Internal Medicine and Microbiology, University of Texas Southwestern Medical School, Dallas, Texas 75390, USA..
robert.munford@utsouthwestern.edu
CONTRACT NUMBER: AI8188 (NIAID)
SOURCE: Journal of endotoxin research, (2005) Vol. 11, No. 2, pp. 69-84. Ref: 166
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 14 Jun 2005
Last Updated on STN: 3 Aug 2005
Entered Medline: 2 Aug 2005

AB Animals that cannot sense endotoxin may die if they are infected by Gram-negative bacteria. Animals that sense endotoxin and respond too vigorously may also die, victims of their own inflammatory reactions. The outcome of Gram-negative bacterial infection is thus determined not only by an individual's ability to sense endotoxin and respond to its presence, but also by numerous phenomena that inactivate endotoxin and/or prevent harmful reactions to it. **Endotoxin** sensing requires the **MD-2**/TLR4 recognition **complex** and occurs principally in local tissues and the liver. This review highlights the known detoxification mechanisms, which include: (i) proteins that facilitate LPS sequestration by plasma lipoproteins, prevent interactions between the bioactive lipid A moiety and MD-2/TLR4, or promote cellular uptake via non-signaling pathway(s); (ii) enzymes that deacylate or dephosphorylate lipid A; (iii) mechanisms that remove LPS and Gram-negative bacteria from the bloodstream; and (iv) neuroendocrine adaptations that modulate LPS-induced mediator production or neutralize pro-inflammatory molecules in the circulation. In general, the mechanisms for sensing and detoxifying endotoxin seem to be compartmentalized (local versus systemic), dynamic, and variable between individuals. They may have evolved to confine infection and inflammation to extravascular sites of infection while preventing harmful systemic reactions. Integration of endotoxin sensing and detoxification is essential for successful host defense.

CT Animals
Bacterial Infections: ME, metabolism
*Endotoxins: ME, metabolism
Endotoxins: TO, toxicity
Humans
Lipid A: ME, metabolism
Research Support, N.I.H., Extramural
Research Support, U.S. Gov't, P.H.S.
Reticuloendothelial System: ME, metabolism

L53 ANSWER 17 OF 22 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2004602663 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15472123
TITLE: Potential role of endotoxin as a proinflammatory mediator of atherosclerosis.
AUTHOR: Stoll Lynn L; Denning Gerene M; Weintraub Neal L
CORPORATE SOURCE: Department of Internal Medicine, Division of Cardiovascular Diseases, University of Iowa, Iowa City and The VA Medical Center, IA 52242, USA.. stolll@mail.medicine.uiowa.edu
SOURCE: Arteriosclerosis, thrombosis, and vascular biology, (2004 Dec) Vol. 24, No. 12, pp. 2227-36. Electronic Publication: 2004-10-07. Ref: 175
Journal code: 9505803. E-ISSN: 1524-4636.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 4 Dec 2004
Last Updated on STN: 22 Jun 2005
Entered Medline: 21 Jun 2005

AB Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Although a variety of inflammatory markers (ie, C-reactive protein) have been associated with atherosclerosis and its consequences, it is important to identify principal mediators of the inflammatory responses. One potentially important source of vascular inflammation in atherosclerosis is bacterial endotoxin. Mutations in Toll-like receptor 4 (TLR-4), an integral component of the endotoxin signaling complex, are fairly common in the Caucasian population and have recently been associated with reduced incidence of atherosclerosis and other cardiovascular diseases in some studies. Moreover, epidemiological studies suggest that endotoxemia at levels as low as 50 pg/mL constitutes a strong risk factor for the development of atherosclerosis. Endotoxin concentrations in this range may be produced by a variety of common subclinical Gram-negative infections. In this article, we outline the main elements of the **endotoxin** signaling receptor **complex** that initiates proinflammatory signaling (lipopolysaccharide binding protein [LBP], CD14, TLR-4, and **MD-2**) and discuss how changes in expression of these molecules may affect proatherogenic responses in the vessel wall. We also describe some of the proinflammatory effects of endotoxin that may be relevant to atherosclerosis, and discuss how serum lipoproteins, especially high-density lipoprotein, may modulate endotoxin-induced inflammatory responses. Further, we discuss recent findings suggesting that the lipid-lowering statins may have an additional protective role in blocking at least some of these proinflammatory signaling pathways. Finally, we discuss species diversity with regard to endotoxin signaling that should be considered when extrapolating experimental data from animal models to humans.

CT Animals
*Arteriosclerosis: PA, pathology
*Endotoxins: PH, physiology
Humans
Inflammation: ME, metabolism

L53 ANSWER 18 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:72508 BIOSIS
DOCUMENT NUMBER: PREV200400076152

TITLE: Lysines 128 and 132 enable lipopolysaccharide binding to MD-2, leading to Toll-like receptor-4 aggregation and signal transduction.

AUTHOR(S): Visintin, Alberto; Latz, Eicke; Monks, Brian G.; Espevik, Terje; Golenbock, Douglas T. [Reprint Author]

CORPORATE SOURCE: Dept. of Medicine, University of Massachusetts Medical School, 364 Plantation St., LRB 309, Worcester, MA, 01605, USA
douglas.golenbock@umassmed.edu

SOURCE: Journal of Biological Chemistry, (November 28 2003) Vol. 278, No. 48, pp. 48313-48320. print.
CODEN: JBCHA3. ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 4 Feb 2004
Last Updated on STN: 4 Feb 2004

AB Three cell-surface proteins have been recognized as components of the mammalian signaling receptor for bacterial lipopolysaccharide (LPS): CD14, Toll-like receptor-4 (TLR4), and MD-2. Biochemical and visual studies shown here demonstrate that the role of CD14 in signal transduction is to enhance LPS binding to MD-2, although its expression is not essential for cellular activation. These studies clarify how MD-2 functions: we found that MD-2 enables TLR4 binding to LPS and allows the formation of stable receptor **complexes.** MD-2 must be **bound** to TLR4 on the cell surface before binding can occur. Consequently, TLR4 clusters into receptosomes (many of which are massive) that recruit intracellular toll/IL-1/resistance domain-containing adapter proteins within minutes, thus initiating signal transduction. TLR4 activation correlates with the ability of MD-2 to bind LPS, as MD-2 mutants that still bind TLR4, but are impaired in the ability to bind LPS, conferred a greatly blunted LPS response. These findings help clarify the earliest events of TLR4 triggering by LPS and identify **MD-2** as an attractive target for pharmacological intervention in **endotoxin**-mediated diseases.

IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Immune System (Chemical Coordination and Homeostasis); Metabolism

IT Chemicals & Biochemicals
CD14: cell-surface protein, signal transduction; MD-2: cell-surface protein, lipopolysaccharide binding; Toll-like receptor-4: aggregation, cell-surface protein, signal transduction; lipopolysaccharide; lysine 128; lysine 132

L53 ANSWER 19 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:521649 BIOSIS

DOCUMENT NUMBER: PREV200300510588

TITLE: Lipopolysaccharide interaction with cell surface toll-like receptor 4-MD-2: Higher affinity than that with MD-2 or CD14.

AUTHOR(S): Akashi, Sachiko; Saitoh, Shin-ichiroh; Wakabayashi, Yasutaka; Kikuchi, Takane; Takamura, Noriaki; Nagai, Yoshinori; Kusumoto, Yutaka; Fukase, Koichi; Kusumoto, Shoichi; Adachi, Yoshiyuki; Kosugi, Atsushi; Miyake, Kensuke [Reprint Author]

CORPORATE SOURCE: Division of Infectious Genetics, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo, 108-8639, Japan
kmiyake@ims.u-tokyo.ac.jp

SOURCE: Journal of Experimental Medicine, (October 6 2003) Vol.

198, No. 7, pp. 1035-1042. print.
ISSN: 0022-1007 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Nov 2003
Last Updated on STN: 5 Nov 2003

AB Toll-like receptors (TLRs) are innate recognition molecules for microbial products, but their direct interactions with corresponding ligands remain unclarified. LPS, a membrane constituent of gram-negative bacteria, is the best-studied TLR ligand and is recognized by TLR4 and MD-2, a molecule associated with the extracellular domain of TLR4. Although TLR4-MD-2 recognizes LPS, little is known about the physical interaction between LPS and TLR4-MD-2. Here, we demonstrate cell surface LPS-TLR4-MD-2 **complexes.** CD14 greatly enhances the formation of LPS-TLR4-MD-2 **complexes**, but is not coprecipitated with LPS-TLR4-MD-2 **complexes**, suggesting a role for CD14 in LPS loading onto TLR4-MD-2 but not in the interaction itself between LPS and TLR4-MD-2. A tentative dissociation constant (Kd) for LPS-TLR4-MD-2 **complexes** was apprx3 nM, which is apprx10-20 times lower than the reported Kd for LPS-MD-2 or LPS-CD14. The presence of detergent disrupts LPS interaction with CD14 but not with TLR4-MD-2. E5531, a lipid A antagonist developed for therapeutic intervention of **endotoxin** shock, blocks LPS interaction with TLR4-**MD-2** at a concentration 100 times lower than that required for blocking LPS interaction with CD14. These results reveal direct LPS interaction with cell surface TLR4-MD-2 that is distinct from that with MD-2 or CD14.

IT Major Concepts
Biochemistry and Molecular Biophysics; Immune System (Chemical Coordination and Homeostasis)
IT Parts, Structures, & Systems of Organisms
cell surface; macrophage: blood and lymphatics, immune system
IT Diseases
endotoxin shock: bacterial disease, infectious disease
IT Chemicals & Biochemicals
CD14; E5531; MD-2; Toll-like receptor 4 [TLR4]; Toll-like receptor 4-MD-2 [TLR4-MD2]; lipopolysaccharide [LPS]

L53 ANSWER 20 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:163318 BIOSIS
DOCUMENT NUMBER: PREV200400164077
TITLE: Regulation of interactions of endotoxin with host cells.
AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zarember, Kol A.; Weiss, Jerrold P. [Reprint Author]
CORPORATE SOURCE: Roy J. and Lucille A. Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA, 52242, USA
jerrold-weiss@uiowa.edu
SOURCE: Journal of Endotoxin Research, (2003) Vol. 9, No. 6, pp. 401-408. print.
ISSN: 0968-0519.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Mar 2004
Last Updated on STN: 24 Mar 2004

AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by **endotoxin** requires lipopolysaccharide-binding protein (LBP) and CD14-dependent delivery of **endotoxin** to cells containing **MD-2** and TLR4. We have used metabolically labeled (14C) meningococcal lipooligosaccharide (LOS), purified recombinant endotoxin-binding proteins, and cultured endothelial cells to better

define protein:endotoxin intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:endotoxin **complexes** or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of endotoxin agg (Mrgtoreq20X106) to monomeric (Mrapprx55X103) endotoxin:sCD14 **complexes.** Ordered interaction of LBP (+albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive endotoxin:sCD14 **complex** and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of endotoxin:sCD14 **complexes** and instead yielded aggregates of endotoxin (Mrapprx1-20X106) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of endotoxin selectively recognizes different protein:endotoxin **complexes.** At the outset of infection, the low concentrations of LBP present and absence of extracellular BPI favor formation of pro-inflammatory endotoxin:CD14 **complexes.** The mobilization of LBP and BPI that is triggered by inflammation directs endotoxin for clearance and hence resolution of endotoxin-triggered inflammation.

IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Infection; Toxicology

IT Parts, Structures, & Systems of Organisms
cell

IT Chemicals & Biochemicals
CD14; TLR4 [toll-like receptor 4]; endotoxin: toxin; lipopolysaccharide binding protein [LBP]

L53 ANSWER 21 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:234536 BIOSIS
DOCUMENT NUMBER: PREV200300234536
TITLE: Overexpression of CD14, TLR4, and MD-2 in HEK 293T cells does not prevent induction of in vitro endotoxin tolerance.
AUTHOR(S): Medvedev, Andrei E.; Vogel, Stefanie N. [Reprint Author]
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland, 655 West Baltimore St, 13-009, Baltimore, MD, 21201, USA
svogel@som.umaryland.edu
SOURCE: Journal of Endotoxin Research, (2003) Vol. 9, No. 1, pp. 60-64. print.
ISSN: 0968-0519.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 May 2003
Last Updated on STN: 14 May 2003

AB TLR4 and MD-2 are necessary for conferring cellular responsiveness to LPS. Prior exposure to LPS induces a transient state of cell refractoriness to subsequent LPS re-stimulation, known as 'endotoxin tolerance'. While induction of LPS tolerance has been reported to correlate with down-regulation of cell surface expression of TLR4/MD-2, other mechanisms of LPS tolerance have been revealed that target intracellular intermediates downstream of the TLR4/MD-2 **complex.** In this study, we sought to examine whether **endotoxin** tolerance could be induced under conditions where expression of TLR4 and **MD-2** proteins is not affected by LPS. Human HEK 293T cells are

completely unresponsive to LPS, but acquire high LPS sensitivity following transient transfection with CD14, TLR4, and MD-2 (293T/CD14/TLR4/MD-2 cells), as judged by NF-kappaB activation, ERK 1/2 phosphorylation, and TNF-alpha gene expression. Prior exposure of 293T/CD14/TLR4/MD-2 cells to LPS resulted in a significant decrease of LPS-mediated responses, yet failed to affect expression levels of TLR4 and MD-2. Thus, altered expression and/or function of intracellular mediators downstream of the TLR4/MD-2 **complex** play an important role in mediating LPS tolerance.

IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Infection; Molecular Genetics (Biochemistry and Molecular Biophysics); Toxicology

IT Chemicals & Biochemicals
CD14: overexpression; ERK1; ERK2; LPS [lipopolysaccharide]: toxin; MD-2: overexpression; TLR4 [toll-like receptor 4]: overexpression; endotoxin: toxin

L53 ANSWER 22 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:5626 BIOSIS
DOCUMENT NUMBER: PREV200300005626
TITLE: Dysregulation of LPS-induced toll-like receptor 4-MyD88 complex formation and IL-1 receptor-associated kinase 1 activation in endotoxin-tolerant cells.
AUTHOR(S): Medvedev, Andrei E.; Lentschat, Arnd; Wahl, Larry M.; Golenbock, Douglas T.; Vogel, Stefanie N. [Reprint Author]
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland, Baltimore, MD, 21201-1559, USA svogel@som.umaryland.edu
SOURCE: Journal of Immunology, (November 1 2002) Vol. 169, No. 9, pp. 5209-5216. print.
ISSN: 0022-1767 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 18 Dec 2002
Last Updated on STN: 18 Dec 2002

AB Prior exposure to LPS induces a transient state of cell refractoriness to subsequent LPS restimulation, known as endotoxin tolerance. Induction of LPS tolerance has been reported to correlate with decreased cell surface expression of the LPS receptor **complex**, Toll-like receptor 4 (TLR4)/MD-2. However, other results have underscored the existence of mechanisms of LPS tolerance that operate downstream of TLR4/MD-2. In the present study we sought to delineate further the molecular basis of LPS tolerance by examining the TLR4 signaling pathway in endotoxin-tolerant cells. Pretreatment of human monocytes with LPS decreased LPS-mediated NF-kappaB activation, p38 mitogen-activated protein kinase phosphorylation, and TNF-alpha gene expression, documenting the induction of endotoxin tolerance. FACS and Western blot analyses of LPS-tolerant monocytes showed increased TLR2 expression, whereas TLR4 expression levels were not affected. Comparable levels of mRNA and protein for myeloid differentiation factor 88 (MyD88), IL-1R-associated kinase 1 (IRAK-1), and TNFR-associated factor-6 were found in normal and LPS-tolerant monocytes, while MD-2 mRNA expression was slightly increased in LPS-tolerant cells. LPS induced the association of MyD88 with TLR4 and increased IRAK-1 activity in medium-pretreated cells. In LPS-tolerant monocytes, however, MyD88 failed to be recruited to TLR4, and IRAK-1 was not activated in response to LPS stimulation. Moreover, **endotoxin**-tolerant CHO cells that overexpress human TLR4 and **MD-2** also showed decreased IRAK-1 kinase activity in response to LPS despite the failure of LPS to inhibit cell surface expression of transfected TLR4 and **MD**

-2 proteins. Thus, decreased TLR4-MyD88 **complex** formation with subsequent impairment of IRAK-1 activity may underlie the LPS-tolerant phenotype.

IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Immune System (Chemical Coordination and Homeostasis); Toxicology

IT Parts, Structures, & Systems of Organisms
monocytes: blood and lymphatics, immune system, in-vitro culture

IT Chemicals & Biochemicals
Toll-like receptor 4: endotoxin tolerant cell complexing dysregulation, entotoxin-induced myeloid differentiation factor 88 complexing; interleukin-1 receptor-associated kinase 1: defective endotoxin tolerant cell activation; lipopolysaccharide [endotoxin]: monocyte tolerance

L54 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2005:1207627 CAPLUS
DOCUMENT NUMBER: 143:458453
TITLE: Biochemical and Functional Characterization of Membrane Blebs Purified from Neisseria meningitidis Serogroup B
AUTHOR(S): Post, Deborah M. B.; Zhang, DeSheng; Eastvold, Joshua S.; **Teghanemt, Athmane**; Gibson, Bradford W.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine and the Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, 52242, USA
SOURCE: Journal of Biological Chemistry (2005), 280(46), 38383-38394
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Studies with purified aggregates of endotoxin have revealed the importance of lipopolysaccharide-binding protein (LBP)-dependent extraction and transfer of individual endotoxin mols. to CD14 in Toll-like receptor 4 (TLR4)-dependent cell activation. Endotoxin is normally embedded in the outer membrane of intact Gram-neg. bacteria and shed membrane vesicles ("blebs"). However, the ability of LBP and CD14 to efficiently promote TLR4-dependent cell activation by membrane-associated endotoxin has not been studied extensively. In this study, the authors used an acetate auxotroph of Neisseria meningitidis serogroup B to facilitate metabolic labeling of bacterial endotoxin and compared interactions of purified endotoxin aggregates and of membrane-associated endotoxin with LBP, CD14, and endotoxin-responsive cells. The endotoxin, phospholipid, and protein composition of the recovered blebs indicate that the blebs derive from the bacterial outer membrane. Proteomic anal. revealed an unusual enrichment in highly cationic (pI > 9) proteins. Both purified endotoxin aggregates and blebs activate monocytes and endothelial cells in a LBP-, CD14-, and TLR4/MD-2-dependent fashion, but the blebs were 3-10-fold less potent when normalized for the amount of endotoxin added. Differences in potency correlated with differences in efficiency of LBP-dependent delivery to and extraction of endotoxin by CD14. Both membrane phospholipids and endotoxin are extracted by LBP/soluble CD14 (sCD14) treatment, but only **endotoxin**·sCD14 reacts with **MD-2** and activates cells.

These findings indicate that the proinflammatory potency of endotoxin may be regulated not only by the intrinsic structural properties of endotoxin but also by its association with neighboring mols. in the outer membrane.

CT Human
CT CD14 (antigen)
CT Proteins
CT Proteins
CT Receptors
CT Monocyte
CT Proteins
CT Blood vessel
CT Neisseria meningitidis
CT Lipopolysaccharides
CT Proteins
CT Cell activation
CT Fatty acids, analysis
CT Phospholipids, analysis
CT Porins
CT Endothelium
CT Organelle

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2004:686516 CAPLUS
DOCUMENT NUMBER: 141:308867
TITLE: **Endotoxin** responsiveness of human airway epithelia is limited by low expression of **MD-2**
AUTHOR(S): Jia, Hong Peng; Kline, Joel N.; Penisten, Andrea; Apicella, Michael A.; **Gioannini, Theresa L.**; Weiss, Jerrold; McCray, Paul B., Jr.
CORPORATE SOURCE: Department of Pediatrics, Carver College of Medicine, University of Iowa and Iowa City Veterans Administration, Iowa City, IA, USA
SOURCE: American Journal of Physiology (2004), 287(2, Pt. 1), L428-L437
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The expression of inducible antimicrobial peptides, such as human β-defensin-2 (HBD-2) by epithelia, comprises a component of innate pulmonary defenses. We hypothesized that HBD-2 induction in airway epithelia is linked to pattern recognition receptors such as the Toll-like receptors (TLRs). We found that primary cultures of well-differentiated human airway epithelia express the mRNA for TLR-4, but little or no MD-2 mRNA, and display little HBD-2 expression in response to treatment with purified endotoxin ± LPS binding protein (LBP) and soluble CD14. Expression of endogenous MD-2 by transduction of airway epithelial cells with an adenoviral vector encoding MD-2 or extracellular addition of recombinant MD-2 both increased the responses of airway epithelia to endotoxin + LBP and sCD14 by > 100-fold, as measured by NF-κB-luciferase activity and HBD-2 mRNA expression. MD-2 mRNA could be induced in airway epithelia by exposure of these cells to specific bacterial or host products (e.g., killed Haemophilus influenzae, the P6 outer membrane protein from H. influenzae, or TNF-α + IFN-γ). These findings suggest that MD-2, either coexpressed with TLR-4 or secreted when produced in excess of TLR-4 from neighboring cells, is required for airway epithelia to respond sensitively to endotoxin. The

regulation of MD-2 expression in airway epithelia and pulmonary macrophages may serve as a means to modify endotoxin responsiveness in the airway.

CT Proteins
CT Receptors
CT Human
CT Toxins
CT Respiratory system
CT Epithelium

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2004:64598 CAPLUS
DOCUMENT NUMBER: 140:269456
TITLE: Regulation of interactions of endotoxin with host cells
AUTHOR(S): **Gioannini, Theresa L.; Teghanemt, Athmane**; Zarember, Kol A.; Weiss, Jerrold P.
CORPORATE SOURCE: Departments of Internal Medicine, Division of Infectious Diseases and The Inflammation Program, Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2003), 9(6), 401-408
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by endotoxin requires lipopolysaccharide-binding protein (LBP) and CD14-dependent delivery of **endotoxin** to cells containing **MD-2** and TLR4. We have used metabolically labeled [14C] meningococcal lipooligosaccharide (LOS), purified recombinant endotoxin-binding proteins, and cultured endothelial cells to better define protein:endotoxin intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:endotoxin complexes or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of endotoxin agg (Mr≥20 + 106) to monomeric (Mr.apprx.55 + 103) endotoxin:sCD14 complexes. Ordered interaction of LBP (+ albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive endotoxin:sCD14 complex and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of endotoxin:sCD14 complexes and instead yielded aggregates of endotoxin (Mr.apprx.1-20 + 106) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of endotoxin selectively recognizes different protein: endotoxin complexes. At the outset of infection, the low concns. of LBP present and absence of extracellular BPI favor formation of pro-inflammatory endotoxin:CD14 complexes. The mobilization of LBP and BPI that is triggered by inflammation directs endotoxin for clearance and hence resolution of endotoxin-triggered inflammation.

CT Proteins
CT Cell activation
CT Human
CT Inflammation
CT Molecular association

CT Receptors
CT Lipopolysaccharides
CT Blood vessel
CT Immunity
CT Proteins
CT Albumins, biological studies
CT CD14 (antigen)
CT Endothelium
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:426231 CAPLUS
DOCUMENT NUMBER: 142:480799
TITLE: Preparation of complexes of **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**
INVENTOR(S): Weiss, Jerrold P.; **Gioannini, Theresa L.**; **Teghanemt, Athamane**; **Subramanian, Ramaswamy**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 34 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005106179	A1	20050519	US 2003-715876	20031117
WO 2005049067	A1	20050602	WO 2004-US38375	20041117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-715876 A 20031117
AB The disclosed invention provides purified water soluble complexes of **endotoxin** and **MD-2.** The invention also provides a method for making the complexes of the invention and a method for isolating complexes of the invention. Also provided are the method of using the complexes of the invention, e.g. method to increase or inhibit TLR4 receptor-dependent activation of cells by endotoxin in vitro or in vivo. Methods using complexes with mutant endotoxin are useful to decrease undesirable endotoxin-mediated inflammation. Methods using complexes with wild-type endotoxin are of use in promoting innate immunity and as immune adjuvants. The results of one example demonstrate that in primary cultures of human airway epithelia TLR4, but little or no MD-2 is expressed, so the cells are relatively unresponsive to added endotoxin. However, the cell responsiveness to endotoxin is markedly amplified by either the endogenous expression or exogenous addition of MD-2.
CT CD14 (antigen)

CT Animal cell line
CT Proteins
CT Receptors
CT Immunostimulants
CT Glycolipids
CT Drug delivery systems
CT Toxins
CT Toxins
CT Respiratory system
CT Immunity
CT Cell activation
CT Escherichia
CT Escherichia coli
CT Francisella
CT Francisella tularensis
CT Haemophilus
CT Haemophilus influenzae
CT Neisseria
CT Neisseria meningitidis
CT Pseudomonas
CT Pseudomonas aeruginosa
CT Salmonella
CT Salmonella typhimurium
CT Anti-inflammatory agents
CT Human
CT Epithelium

L54 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:1016771 CAPLUS
DOCUMENT NUMBER: 143:304613
TITLE: Molecular Basis of Reduced Potency of Underacylated **Endotoxins**
AUTHOR(S): **Teghanemt, Athmane**; Zhang, DeSheng; Levis, Erika N.; Weiss, Jerrold P.; **Gioannini, Theresa L.**
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA, 52241, USA
SOURCE: Journal of Immunology (2005), 175(7), 4669-4676
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxins depends on sequential endotoxin-protein and protein-protein interactions with LPS-binding protein, CD14, myeloid differentiation protein 2 (MD-2), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated endotoxins (i.e., tetra- or penta- vs. hexa-acylated) is determined by post-CD14 interactions. To better define the mol. basis of the differences in agonist potency of endotoxins differing in fatty acid acylation, the authors compared endotoxins (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB meningococcal strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of endotoxin:protein and endotoxin:cell interactions, the endotoxins were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric complexes with MD-2 in an LPS-binding protein- and CD14-dependent manner with similar efficiency. However, msbB LOS:MD-2 and acyloxyacylhydrolase-treated LOS:MD-2 were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:MD-2 and partially antagonized the action of wt

LOS:MD-2. These findings suggest that underacylated endotoxins produce decreased TLR4-dependent cell activation by altering the interaction of the **endotoxin:MD-2** complex with TLR4 in a way that reduces receptor activation. Differences in potency among these endotoxin species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin:MD-2** complexes.

CT Proteins
CT Receptors
CT Toxins
CT Lipopolysaccharides
CT Structure-activity relationship
CT Cell activation
CT CD14 (antigen)
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:489362 CAPLUS
DOCUMENT NUMBER: 143:95745
TITLE: Monomeric **endotoxin**:protein complexes are essential for TLR4-dependent cell activation
AUTHOR(S): **Gioannini, T. L.; Teghanemt, A.**; Zhang, De S.; Levis, E. N.; Weiss, J. P.
CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa and the Veterans' Administration Medical Center, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2005), 11(2), 117-123
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxin depends on sequential endotoxin-protein and protein-protein interactions with LBP, CD14, MD-2 and TLR4. LBP and CD14 combine, in an albumin-dependent fashion, to extract single endotoxin mols. from purified endotoxin aggregates (Eagg) or the bacterial outer membrane and form monomeric endotoxin:CD14 complexes that are the preferred presentation of **endotoxin** for transfer to **MD-2.** Endotoxin in endotoxin:CD14 is readily transferred to MD-2, again in an albumin-dependent manner, to form monomeric **endotoxin:MD-2** complex. This monomeric **endotoxin**:protein complex (**endotoxin:MD-2**) activates TLR4 at picomolar concns., independently of albumin, and is, therefore, the apparent ligand in endotoxin-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated forms of meningococcal endotoxin (LOS) react similarly with LBP, CD14, and **MD-2** to form **endotoxin:MD-2** complexes. However, tetra- and penta-acylated LOS:MD-2 complexes are less potent TLR4 agonists than hexa-acylated LOS:MD-2. This is mirrored in the reduced activity of tetra-, penta- vs. hexa-acylated LOS aggregates (LOSagg) + LBP toward cells containing mCD14, MD-2, and TLR4. Therefore, changes in agonist potency of under-acylated meningococcal LOS are determined by differences in properties of monomeric **endotoxin:MD-2.**

CT Proteins
CT Receptors
CT Blood vessel
CT Toxins
CT Glycolipids

CT Cell activation
CT CD14 (antigen)
CT Albumins, biological studies
CT Endothelium
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:292853 CAPLUS
DOCUMENT NUMBER: 140:401625
TITLE: Isolation of an **endotoxin-MD-2** complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations
AUTHOR(S): **Gioannini, Theresa L.**; **Teghanemt, Athmane**; Zhang, DeSheng; Coussens, Nathan P.; Dockstader, Wendie; Ramaswamy, S.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, and Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA, 52242, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(12), 4186-4191 CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Host proinflammatory responses to minute amts. of endotoxins derived from many Gram-neg. bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and MD-2. Optimal sensitivity to endotoxin requires an ordered series of endotoxin-protein and protein-protein interactions. At substoichiometric concns., LBP facilitates delivery of endotoxin aggregates to soluble CD14 (sCD14) to form monomeric endotoxin-sCD14 complexes. Subsequent interactions of endotoxin-sCD14 with TLR4 and/or MD-2 have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive **endotoxin-MD-2** complex generated by treatment of **endotoxin**-sCD14 with recombinant **MD-2.** Efficient generation of this complex occurred at picomolar concns. of endotoxin and nanogram per mL doses of **MD-2** and required presentation of **endotoxin** to **MD-2** as a monomeric **endotoxin**-CD14 complex. TLR4-dependent delivery of endotoxin to human embryonic kidney (HEK) cells and cell activation at picomolar concns. of endotoxin occurred with the purified **endotoxin-MD-2** complex, but not with purified endotoxin aggregates with or without LBP and/or sCD14. The presence of excess **MD-2** inhibited delivery of **endotoxin-MD-2** to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concns. of endotoxin occurs by sequential interaction and transfer of **endotoxin** to LBP, CD14, and **MD-2** and simultaneous engagement of **endotoxin** and TLR4 by **MD-2.**
CT Animal cell line
CT Proteins
CT Proteins
CT Receptors
CT CD14 (antigen)
CT Toxins

CT Human
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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